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Fatty liver index is associated with albuminuria and chronic kidney disease: a real-world evidence study

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Fatty liver index is associated with albuminuria and chronic kidney disease: a real-world evidence study

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All authors believe that the manuscript represents valid work and have reviewed and approved the final version. The work has not been published previously, and not under consideration for publication elsewhere, in part or in whole.

The author contribution lists

Conceived and designed the experiments: Y. L. and K. S.

Performed the experiments: F. L., Y. Q., W. F., C. C., K. S. and D. L.

Analyzed the data: K. S. and M. R.

Wrote the manuscript: K.S. and D. L.

Data Sharing Statement

The work described was original research that has not been published previously, and not under consideration for publication elsewhere, in part or in whole. All authors believe that the manuscript represents valid work and have reviewed and approved the final version. Main document data and additional unpublished data from the study are available by sending Email to lizyhenu@163.com with proper purposes.

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Conflict of interests

The authors have declared that no competing interests exist.

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ABSTRACT

Objectives: The effects of lipid metabolism disorder on the renal damage have drawn much attention. By using the fatty liver index (FLI) as a validated indicator of hepatic steatosis, this study aims at provide insight about the possible links between fatty liver and development of chronic kidney disease (CKD).

Setting: level of care: primary.

Participants: We performed a population-based study in 9,436 subjects aged 40 years or older.

Primary and secondary outcome measures: FLI is calculated by using an algorithm based on body mass index (BMI), waist circumference (WC), triglycerides (TG) and γ-glutamyltransferase (γ-GGT). Increased urinary albumin excretion was defined according to the urinary albumin-to-creatinine ratio ranges greater or equal than 30 mg/g. CKD was defined as estimated glomerular filtration rate (eGFR) less than 60 mL/min per 1.73 m² or presence of albuminuria.

Results: There were 620 (6.6%) subjects categorized as increased urinary albumin excretion and 753 (8.0%) subjects categorized as CKD. Participants with higher FLI had increased age, blood pressure, low-density lipoprotein cholesterol, fasting plasma glucose, fasting insulin and decreased eGFR level. Prevalence of increased urinary albumin excretion and CKD tended to increase with the elevated FLI quartiles. In logistic regression analysis, compared with subjects in the lowest quartile of FLI, the adjusted odds ratios (ORs) in the highest quartile was 2.30 [95% confidence interval

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(CI), 1.36 - 3.90] for increased urinary albumin excretion and 1.93 (95% CI, 1.18 - 3.15) for CKD.

Conclusion: Hepatic steatosis evaluating by FLI is independently associated with increased urinary albumin excretion and prevalence of CKD in middle-aged and elderly Chinese.

Keywords: Fatty liver index; Hepatic steatosis; Increased urinary albumin excretion; Chronic kidney disease

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Introduction

As directly affects the global burden of cardiovascular disease mortality, chronic kidney disease (CKD) has become one of the leading public health problem world-wide [1]. The most recent national survey in 2012 reported that the prevalence of CKD was 10.8%, representing an estimated 119.5 million patients in China with chronic kidney damage [2]. In addition to CKD, an increasing number of studies have provided substantial evidence of albuminuria as a risk factor for future cardiovascular events [3]. Both renal and cardiovascular diseases sharing similar traditional risk factors, such as lipid metabolism disorder, which could have particularly broad implications for the outcome of cardiovascular morbidity and mortality.

Association of hepatic steatosis with CKD development and its impact on the reduction of the estimated glomerular filtration rate (eGFR) have been extensively investigated over the past decade [4]. The substantial evidence linked hepatic steatosis to the increased risk and severity of CKD, which may be a target for the prevention and treatment of the disease [5]. As a convenient scoring system for the presence of hepatic lipid deposits, the fatty liver index (FLI) is a surrogate steatosis biomarker developed in a cohort of patients from the general population [6]. Compared with other techniques for evaluating hepatic steatosis, FLI is simple to obtain as body mass index (BMI), waist circumference (WC), triglycerides (TG) and γ-glutamyltransferase (γ-GGT) are routine measurements in clinical practice. Previous studies have demonstrated that FLI could determine fatty liver disease, incident type 2 diabetes and

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incident hypertension with considerable accuracy [6-8]. Moreover, FLI is associated with insulin resistance early atherosclerosis and risk of coronary heart disease, which could help physicians early detect subjects of greater cardiovascular risk and select patients for intensified lifestyle counseling [9 10].

Clarified the association of FLI with albuminuria and prevalent CKD would probably shed light on the prevention and preemptive treatment of related diseases. Recently, a cross-sectional study was conducted to investigate the association between FLI and CKD by recruiting adults undergoing a health check-up [11]. However, by including only 731 subjects, the study did not evaluate the association between FLI and albuminuria, either. Therefore, we analyzed data from a community-based Chinese population to comprehensively look into the relationship of FLI with both increased urinary albumin excretion and CKD.

Subjects and methods

Study population and design

We performed a cross-sectional study in a community in Guangzhou, China from June to November, 2011. The study population was from the REACTION study and details of this study have been published previously [12-14]. During the recruiting phase, a total of 10,104 residents aged 40 years or older were invited to participate by examination notices or home visits. In total, 9,916 subjects signed the consent form and agreed to participate in the survey, and the participation rate was 98.1%. The subjects who failed to provided information (BMI: n=206; WC: n=62; TG: n=23;

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γ-GGT: n=38; or urinary albumin-to-creatinine ratio [ACR]: n=149) were excluded from the analyses. Accordingly, a total of 9,438 eligible individuals were included in the final data analyses. The study protocol was approved by the Institutional Review Board of the Sun Yat-sen Memorial Hospital affiliated to Sun Yat-sen University and was in accordance with the principle of the Helsinki Declaration II. Written informed consent was obtained from each participant before data collection.

Clinical and biochemical measurements

We collected information on lifestyle factors, sociodemographic characteristics and family history by using a standard questionnaire. Smoking or drinking habit was classified as 'never', 'current' (smoking or drinking regularly in the past 6 months) or 'ever' (cessation of smoking or drinking more than 6 months) [15]. A short form of the International Physical Activity Questionnaire (IPAQ) was used to estimate physical activity at leisure time by adding questions on frequency and duration of moderate or vigorous activities and walking [16]. Separate metabolic equivalent hours per week (MET-h/week) were calculated for evaluation of total physical activity.

All participants completed anthropometrical measurements with the assistance of trained staff by using standard protocols. Three times consecutively blood pressure measurements by the same observer with a 5-minute interval were obtained by an automated electronic device (OMRON, Omron Company, China). The average of three measurements of blood pressure was used for analysis. Body height and body weight were recorded to the nearest 0.1 cm and 0.1 kg while participants were wearing light indoor clothing without shoes. BMI was calculated as weight in

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kilograms divided by height in meters squared (kg/m^2). Obesity was defined as BMI equal or greater than 28 and overweight was defined as BMI equal or greater than 24 and less than 28. WC was measured at the umbilical level with participant in standing position, at the end of gentle expiration.

Venous blood samples were collected for laboratory tests after an overnight fasting of at least 10 hours. Measurement of fasting plasma glucose (FPG), fasting serum insulin, TG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine, γ-GGT, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was done using an autoanalyser (Beckman CX-7 Biochemical Autoanalyser, Brea, CA, USA).

As surrogate marker of hepatic steatosis, FLI was analyzed based on BMI, WC, TG, and γ-GGT, which has been validated against liver ultrasound in the general population and has been proven accurate in detecting fatty liver [6 10]. FLI is calculated as: $FLI = (e^{0.953 * \log e(TG) + 0.139 * BMI + 0.718 * \log e(GGT) + 0.053 * WC - 15.745}) / (1 + e^{0.953 * \log e (TG) +0.139 * BMI + 0.718 * \log e (GGT) + 0.053 * WC - 15.745}) * 100$. The abbreviated Modification of Diet in Renal Disease (MDRD) formula recalibrated for Chinese population was used to calculate estimated glomerular filtration rate (GFR) expressed in mL/min per 1.73 m^2 using a formula of $eGFR = 175 \times [\text{serum creatinine} \times 0.011]^{-1.234} \times [\text{age}]^{-0.179} \times [0.79 \text{ if female}]$, where serum creatinine was expressed as μmol/L [17]. Diabetes was diagnosed according to the 1999 World Health Organization diagnostic criteria [18].

Definition of increased urinary albumin excretion and chronic kidney disease.

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Definitions of abnormalities in albumin excretion were according to the latest guidelines of American Diabetes Association's Standards of Medical Care [19]. The first morning spot urine samples were collected for assessing the ACR. Urine albumin and creatinine were measured by chemiluminescence immunoassay (Siemens Immulite 2000, United States) and the Jaffe' s kinetic method (Biobase-Crystal, Jinan, China) on the automatic analyzer, respectively. ACR was calculated by dividing the urinary albumin concentrations by the urinary creatinine concentrations and expressed in mg/g. Increased urinary albumin excretion was defined according to the ACR ranges greater or equal than 30 mg/g. Chronic kidney disease (CKD) was defined as eGFR less than 60 mL/min per 1.73 m^2 or presence of albuminuria (ACR greater or equal than 30 mg/g).

Statistical analysis

Statistical analysis was performed using SAS version 9.2 (SAS Institute Inc, Cary, NC, USA). Continuous variables were presented as means ± standard deviation (SD) except for skewed variables, which were presented as medians (interquartile ranges). Categorical variables were expressed as numbers (proportions). FLI, FPG, TG, ALT, AST, γ-GGT and MET-h/week were logarithmically transformed before analysis due to a non-normal distribution. FLI was presented as quartiles and linear regression analysis was used to test for trend across groups. Differences among groups were tested by one-way ANOVA and *post hoc* comparisons were performed by using Bonferroni correction. Comparisons between categorical variables were performed

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with the χ^2 test.

Pearson's correlations were performed to test the correlations between FLI and the risk factors for kidney disease. Variables significant at $P < 0.20$ in Pearson's correlations were put into the multivariate stepwise linear regression models to identify factors that independently associated with FLI. We analyzed the impact of FLI on the prevalence of increased urinary albumin excretion and CKD. The unadjusted and multivariate-adjusted logistic regression analysis was used to assess the risk of prevalent increased urinary albumin excretion and CKD in relation to each quartile increase in FLI level. Variables considered as potential covariates and significant in the stepwise linear regression were put into multivariate-adjusted logistic regression analysis. Model 1 is unadjusted. Model 2 is adjusted for age. Model 3 is adjusted for age, sex, BMI, WC, current smoking status, current drinking status, physical activity, SBP, DBP, TG, LDL-C, fasting insulin, ALT, AST and γ-GGT. Odds ratios (OR) and the corresponding 95% confidence intervals (95% CI) were calculated. Relationship of FLI level with albuminuria and CKD were also explored in subgroups stratified by gender (men/women), age (≥ 60/< 60 years), degree of obesity (normal/overweight/obesity), current smoking (yes/no), current drinking (yes/no), hypertension (yes/no) and diabetes (yes/no). Tests for interaction were performed with including simultaneously each strata factor, the quartiles of FLI level and the respective interaction terms (strata factor multiplied by quartiles of FLI level) in the models.

All statistical tests were two-sided, and a P value < 0.05 was considered

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statistically significant.

Results

Clinical characteristics of the study population

Among the 9,436 enrolled individuals, the mean age was 55.9 ± 8.0 years. The median FLI was 19.1 with interquartile range 8.6 to 37.4. There were 620 (6.6%) subjects categorized as increased urinary albumin excretion and 753 (8.0%) subjects categorized as CKD, respectively. Table 1 shows the clinical and biochemical characteristics of the participants according to FLI quartiles. Participants with higher FLI level had elevated age, BMI, WC, SBP, DBP, TG, TC, LDL-C, FPG, fasting insulin, ALT, AST, γ - GGT and higher proportions of current smokers and current drinkers (all P for trend < 0.0001). Those with higher FLI level also associated with decreased HDL-C and eGFR (all P for trend < 0.0001).

Associations between FLI and metabolic risk factors

Analysis of Pearson's correlation showed that age, sex, BMI, WC, SBP, DBP, TG, TC, HDL-C, LDL-C, FPG, fasting insulin, ALT, AST, γ-GGT and eGFR were significantly correlated with FLI level. Further multivariate stepwise linear regression showed that age, sex, BMI, WC, SBP, DBP, TG, LDL-C, fasting insulin, ALT, AST and γ-GGT were independent determinants for FLI level (Table 2).

Associations of FLI with increased urinary albumin excretion and CKD

As shown in Figure.1A, from the lowest quartile to the highest quartile of FLI level, the prevalence of increased urinary albumin excretion was 3.64%, 4.83%,

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6.23% and 11.57%, respectively (P for trend < 0.0001). Strikingly, the prevalence of CKD also tended to increase with the elevated FLI quartile (Figure.1B, P for trend < 0.0001). As shown in Table 3, compared with participants in quartile 1 of FLI, univariate logistic regression analysis showed that participants in quartile 2, quartile 3 and quartile 4, respectively, have a significant correlation with increased odds of increased urinary albumin excretion and CKD (all P for trend < 0.0001). In multivariate logistic regression analyses (Model 3), the ORs of increased urinary albumin excretion for increasing FLI quartiles were 1.00 (reference), 0.96 (95% CI 0.66 - 1.39), 1.17 (95% CI 0.77 - 1.77) and 2.30 (95% CI 1.36 - 3.90). Similarly, the ORs of CKD for increasing FLI quartiles in Model 3 were 1.00 (reference), 1.00 (95% CI 0.71 - 1.40), 1.03 (95% CI 0.70 - 1.51) and 1.93 (95% CI 1.18 - 3.15), respectively (Table 3).

Subgroups analysis of FLI with increased urinary albumin excretion and CKD

As shown in Figure. 2 & 3, the associations of FLI level with increased urinary albumin excretion and CKD were not consistently the same in subgroups analyses. Significant relationship of FLI level with both increased urinary albumin excretion and CKD were detected in women, younger subjects (age less than 60 years), overweight subjects, non-current smokers, non-current drinkers and in those with hypertension or with diabetes (all P < 0.05). In the subgroups analysis, no statistically significance of interaction term between quartiles of FLI and each strata factor was detected.

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Discussion

We evaluated the association between hepatic steatosis and kidney disease in a large population of middle-age Chinese subjects from the REACTION study. Presence of fatty liver assessed by FLI was associated with increased urinary albumin excretion and reduction of the eGFR in the present study. The association was independent of potential confounding risk factors. To our current knowledge, this is the largest population-based study to explore the association of FLI with both albuminuria and CKD. Early intervention is of great importance for albuminuria and CKD, the present findings may just give insights into lipid metabolism for prevention and early detection of the diseases.

The best method for an accurate assessment and diagnosis of hepatic steatosis is histologic analysis of biopsies [20]. However, it is uneconomical to conduct liver biopsies especially by the fact of our large sample population. Hepatic ultrasonic examination is widely used in clinical practice and epidemiological studies in detecting fatty infiltration of the liver [21 22]. However, the noninvasive technique is not sensitive enough to detect mild steatosis and does not allow precise quantification of severity of fatty degeneration in hepatic tissue [23]. As another surrogate marker of histological fatty liver, FLI is defined as the accumulation of excessive liver fat [24]. Based on the former researches, FLI has been proven accurate in detecting fatty liver against liver ultrasound and demonstrating the presence of hepatic fat against magnetic resonance spectroscopy [6 9 10]. The superiority of this non-invasive assessment techniques is that a higher score will indicate a higher rate of liver fatty

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degeneration. However, optimal cut-off point of the FLI for evaluating liver fatty infiltration should be considered as it varied according to the study population [25 26]. Through the results of our research in Chinese subjects, further studies are therefore needed to externally discuss the optimal cut-off point of the FLI for predicting hepatic steatosis.

Detection and prevention of kidney disease progression and urinary albumin excretion is difficult to process in the early stage. Dyslipidemia is increasingly recognized as important pathogenic mechanism in deterioration of renal function. Recently, we conducted a clinical investigation to assess the associations of routine lipid measures with kidney disease in the same cohort. In the study, discordant associations of lipid parameters with renal insufficiency was detected while TG to HDL-C ratio is a better marker for evaluating increased urinary albumin excretion and CKD [27]. As one of the phenotype of dyslipidemia, the pathogeneses of hepatic steatosis is closely related to kidney disease with regard to insulin resistance and chronic inflammation [28]. Hepatokines, which are proteins secreted by hepatocytes, have been found to link to the induction of metabolic phenotypes through inter-organ communication based on recent studies [29]. Because of the high prevalence and burden of the fatty liver disease, it is important to identify which patients are most likely to be exposed to early stage renal injury [30]. Consequently, we closely monitor the association of the hepatic steatosis predict by FLI with prevalent increased urinary albumin excretion and CKD.

Consistent with our findings, a previous study reported that hepatic steatosis

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evaluated by FLI might contribute to CKD development [11]. Elevated albuminuria is well known to be associated with increased risk for early diabetes renal damage, however, the identification and classification of kidney disease was assessed only by eGFR in that study. Moreover, 731 adults that underwent routine health evaluations were included in that study and the small sample size cannot better represent the whole population. By totally including 9,438 subjects and adopting both albuminuria and eGFR for renal damage assessment, the data in our study demonstrated that the FLI is associated with kidney disease, which might be an efficient screening indicator for the early prevention of related diseases.

Some limitations of the study must be noted. Firstly, owing to the observational design of the current study, we should cautiously interpret the present findings as no causal inference can be drawn. Further prospective studies are therefore needed to determine the precise relationship between FLI and risk of renal diseases. Secondly, by including only Chinese subjects, the results of the present study might not be representative of other ethnic groups, especially for those in the developed or undeveloped countries. To some extent, however, the present study of Chinese population was still a convenience sample and selection bias is inevitable. Thirdly, when evaluating the findings of the present study, the results should be interpreted cautiously due to possible bias from using the indirect indicator FLI to assess fatty liver disease. Moreover, the internal accuracy of FLI for evaluation hepatic steatosis should also be validated by using other techniques, before it can be employed for these purposes. Fourthly, we observed that FLI seem to play a different efficiency for

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kidney disease assessment in different stratifications. For example, a significant association of FLI with increased urinary albumin excretion and CKD only in subjects without current alcohol consumption. To better discriminate alcoholic fatty liver disease and non-alcoholic fatty liver disease, further studies need to clearly described the precise exposure of alcohol use by collecting histories of alcohol intake in a quantitative manner. Fifthly, although a spectrum of covariates was included in the adjustment, other potential mediators such as daily energy and protein intake and medicine that influence the renin-angiotensin-system of the subjects, should also be considered in the present study.

In conclusion, by including a large population based cohort, the present study provides evidence that increased FLI is independently associated with prevalence of albuminuria and CKD. Further prospective studies are necessary to verify our findings in external populations.

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Figure legends

Figure. 1 Prevalence of increased urinary albumin excretion and CKD in different quartiles of FLI levels. (A) Increased urinary albumin excretion. (B) CKD.

Figure. 2 Risk of prevalent increased urinary albumin excretion with each quartile increase of FLI levels in different subgroups.

Figure. 3 Risk of prevalent CKD with each quartile increase of FLI levels in different subgroups.

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366

367

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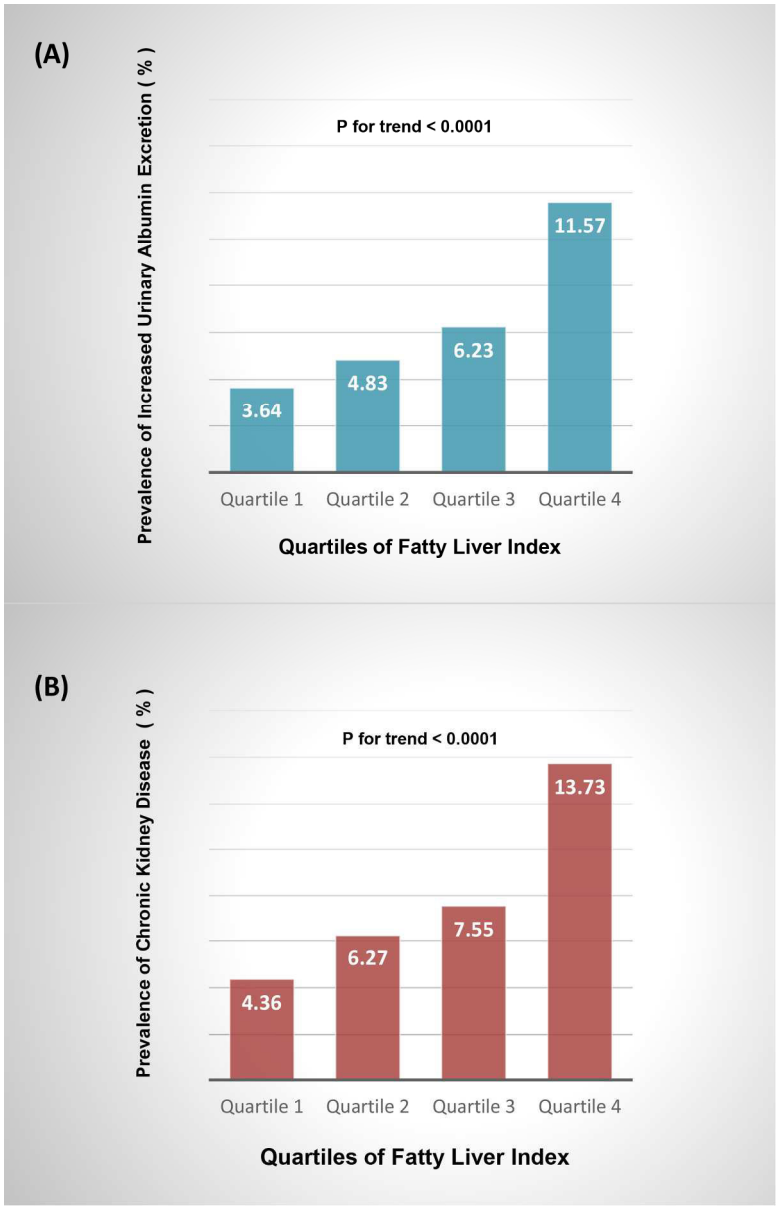

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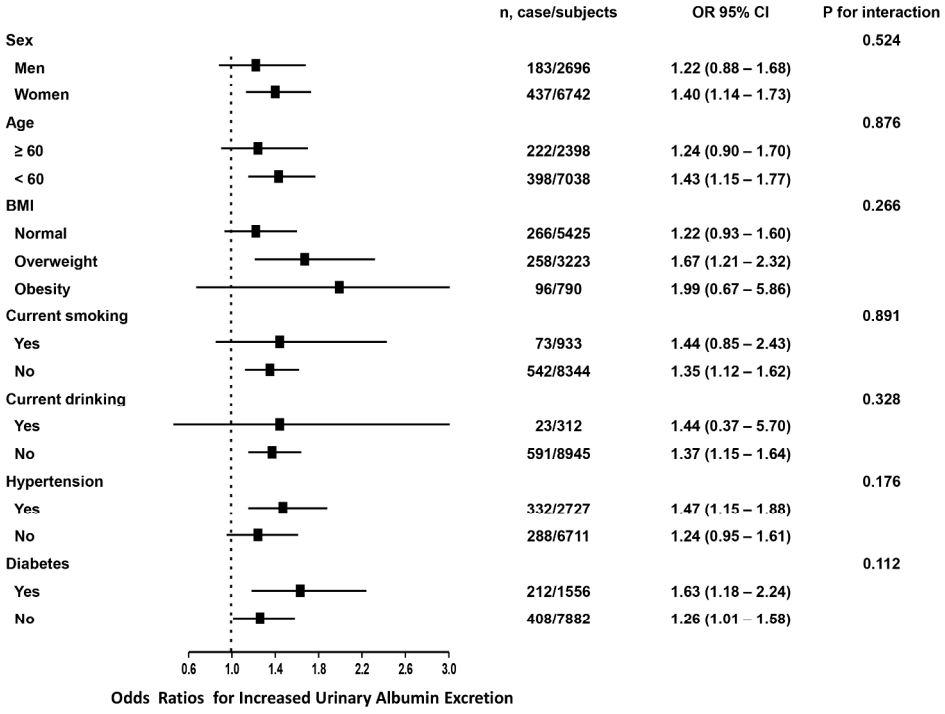

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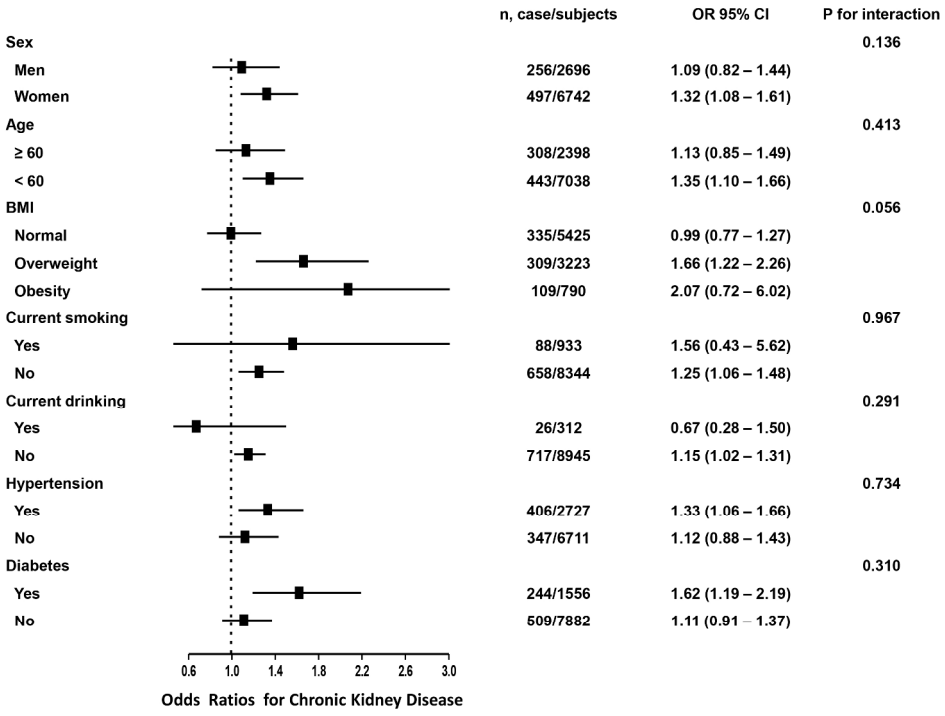

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Table 1. Characteristics of study population by FLI quartiles

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
n (%)	2360 (25.01)	2359 (24.99)	2359 (24.99)	2360 (25.01)	
Fatty liver index	5.00 (3.27 – 6.74)	13.23 (10.76 – 15.99)	26.96 (22.74 – 31.75)	54.71 (45.40 – 68.10)	
Urinary albumin to creatinine ratio (mg/g)	7.65 (5.59 – 11.12)	8.01 (5.64 – 11.71)	8.06 (5.73 – 11.83)*	8.93 (5.96 – 15.01)*	< 0.0001
Age (years)	54.3 ± 7.8	55.8 ± 7.9*	56.5 ± 7.9*	56.9 ± 8.3*	< 0.0001
Male [n (%)]	427 (18.09)	593 (25.17)	701 (29.72)	975 (41.31)	< 0.0001
BMI (kg/m^2)	20.6 ± 2.0	22.9 ± 2.0*	24.4 ± 2.1*	26.8 ± 3.5*	< 0.0001
WC (cm)	72.0 ± 5.8	79.3 ± 5.4*	84.1 ± 5.5*	91.3 ± 8.5*	< 0.0001
SBP (mmHg)	118.6 ± 14.7	124.5 ± 15.9*	128.4 ± 15.8*	132.5 ± 16.1*	< 0.0001
DBP (mmHg)	71.2 ± 9.1	74.2 ± 9.3*	76.5 ± 9.4*	79.3 ± 9.8*	< 0.0001
Current smoking [n (%)]	169 (7.3)	202 (8.7)	227 (9.8)	335 (14.4)	< 0.0001
Current drinking [n (%)]	57 (2.5)	70 (3.0)	68 (2.9)	117 (5.1)	< 0.0001
TG (mmol/L)	0.85 (0.69 – 1.07)	1.12 (0.90 – 1.43)*	1.49 (1.13 – 1.94)*	2.10 (1.56 – 3.01)*	< 0.0001
TC (mmol/L)	4.79 ± 1.24	5.16 ± 1.22*	5.35 ± 1.13*	5.54 ± 1.17*	< 0.0001
HDL-C (mmol/L)	1.45 ± 0.41	1.37 ± 0.35*	1.29 ± 0.31*	1.19 ± 0.28*	< 0.0001

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LDL-C (mmol/L)	2.82 ± 0.90	3.19 ± 0.94*	3.31 ± 0.91*	3.28 ± 0.95*	< 0.0001
FPG (mmol/L)	5.23 (4.89 – 5.61)	5.33 (4.95 – 5.80)*	5.47 (5.05 – 5.96)*	5.73 (5.23 – 6.42)*	< 0.0001
Fasting insulin (μIU/ml)	5.10 (3.90 – 6.50)	6.50 (5.00 – 8.40)*	7.90 (6.10 – 10.30)*	10.50 (7.80 – 13.70)*	< 0.0001
ALT (U/L)	10.0 (8.0 – 14.0)	12.0 (9.0 – 16.0)*	13.0 (10.0 – 17.0)*	17.0 (12.0 – 24.0)*	< 0.0001
AST (U/L)	17.0 (14.0 – 20.0)	18.0 (15.0 – 21.0)*	18.0 (15.0 – 22.0)*	20.0 (17.0 – 25.0)*	< 0.0001
γ-GGT (U/L)	14.0 (11.0 – 17.0)	18.0 (14.0 – 23.0)*	22.0 (17.0 – 29.0)*	31.0 (23.0 – 47.0)*	< 0.0001
eGFR (ml/min per 1.73 m^2)	108.0 ± 25.4	102.5 ± 23.7*	99.9 ± 19.6*	95.5 ± 19.5*	< 0.0001
Physical activity (MET-h/week)	24.0 (10.5 – 49.0)	24.0 (10.5 – 45.0)	23.0 (10.5 – 42.0)	21.0 (10.5 – 42.0)*	0.006

1. Data were means ± SD or medians (interquartile ranges) for skewed variables or numbers (proportions) for categorical variables.
2. P for trend was calculated for the linear regression analysis tests across the groups. P values were for the ANOVA or χ^2 analyses across the groups.
3. *P < 0.05 compared with Quartile 1 of fatty liver index.
4. BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GGT, γ-glutamyltransferase; eGFR, estimated glomerular filtration rate.

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Table 2. Pearson's correlation and stepwise regression analysis of determinants of FLI

	r	P value	Standardized β	P value
Age (years)	0.12	< 0.0001	0.01	0.010
Sex (men=1, women=2)	-0.19	< 0.0001	-0.04	< 0.0001
BMI (kg/m^2)	0.71	< 0.0001	0.30	< 0.0001
WC (cm)	0.78	< 0.0001	0.42	< 0.0001
Physical activity (MET-h/week)	-0.02	0.060	-	-
SBP (mmHg)	0.32	< 0.0001	0.01	0.006
DBP (mmHg)	0.32	< 0.0001	0.01	0.047
TG (mmol/L)	0.68	< 0.0001	0.42	< 0.0001
HDL-C (mmol/L)	-0.26	< 0.0001	-	-
LDL-C (mmol/L)	0.21	< 0.0001	0.06	< 0.0001
FPG (mmol/L)	0.22	< 0.0001	-	-
Fasting insulin (μIU/ml)	0.40	< 0.0001	0.01	0.0002
ALT (U/L)	0.20	< 0.0001	0.05	< 0.0001
AST (U/L)	0.15	< 0.0001	-0.03	< 0.0001
γ-GGT (U/L)	0.35	< 0.0001	0.16	< 0.0001
eGFR (ml/min per 1.73 m^2)	-0.19	< 0.0001	-	-

r, correlation coefficient; β, regression coefficient.

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Table 3. The risk of prevalent albuminuria and CKD according to quartiles of FLI

		Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Increased urinary albumin excretion	Model 1	1	1.34 (1.01 – 1.79)	1.76 (1.34 – 2.31)	3.46 (2.70 – 4.44)	< 0.0001
	Model 2	1	1.29 (0.97 – 1.72)	1.66 (1.27 – 2.19)	3.25 (2.53 – 4.17)	< 0.0001
	Model 3	1	0.96 (0.66 – 1.39)	1.17 (0.77 – 1.77)	2.30 (1.36 – 3.90)	0.001
CKD	Model 1	1	1.47 (1.13 – 1.90)	1.79 (1.39 – 2.30)	3.49 (2.77 – 4.39)	< 0.0001
	Model 2	1	1.39 (1.07 – 1.80)	1.65 (1.28 – 2.12)	3.16 (2.51 – 3.99)	< 0.0001
	Model 3	1	1.00 (0.71 – 1.40)	1.03 (0.70 – 1.51)	1.93 (1.18 – 3.15)	0.012

Data are odds ratios (95% confidence interval). Participants without increased urinary albumin excretion or CKD are defined as 0 and with increased urinary albumin excretion or CKD as 1.

Model 1 is unadjusted.

Model 2 is adjusted for age.

Model 3 is adjusted for age, sex, BMI, WC, current smoking status, current drinking status, physical activity, SBP, DBP, TG, LDL-C, fasting insulin, ALT, AST and γ-GGT.

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Fatty liver index is associated with albuminuria and chronic kidney disease: a population-based study

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Fatty liver index is associated with albuminuria and chronic kidney disease: a population-based study

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Statement of authorship

All authors believe that the manuscript represents valid work and have reviewed and approved the final version. The work has not been published previously, and not under consideration for publication elsewhere, in part or in whole.

The author contribution lists

Conceived and designed the experiments: Y. L. and K. S.

Performed the experiments: F. L., Y. Q., W. F., C. C., K. S. and D. L.

Analyzed the data: K. S. and M. R.

Wrote the manuscript: K.S. and D. L.

Data Sharing Statement

The work described was original research that has not been published previously, and not under consideration for publication elsewhere, in part or in whole. All authors believe that the manuscript represents valid work and have reviewed and approved the final version. Main document data and additional unpublished data from the study are available by sending Email to lizyhenu@163.com with proper purposes.

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Conflict of interests

The authors have declared that no competing interests exist.

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ABSTRACT

Objectives: The effects of lipid metabolism disorder on the renal damage have drawn much attention. By using the fatty liver index (FLI) as a validated indicator of hepatic steatosis, this study aims at provide insight about the possible links between fatty liver and development of chronic kidney disease (CKD).

Setting: hospital.

Participants: We performed a population-based study in 9,436 subjects aged 40 years or older.

Primary and secondary outcome measures: FLI is calculated by using an algorithm based on body mass index (BMI), waist circumference (WC), triglycerides (TG) and γ-glutamyltransferase (γ-GGT). Increased urinary albumin excretion was defined according to the urinary albumin-to-creatinine ratio ranges greater or equal than 30 mg/g. CKD was defined as estimated glomerular filtration rate (eGFR) less than 60 mL/min per 1.73 m² or presence of albuminuria.

Results: There were 620 (6.6%) subjects categorized as increased urinary albumin excretion and 753 (8.0%) subjects categorized as CKD. Participants with higher FLI had increased age, blood pressure, low-density lipoprotein cholesterol, fasting plasma glucose, fasting insulin and decreased eGFR level. Prevalence of increased urinary albumin excretion and CKD tended to increase with the elevated FLI quartiles. In logistic regression analysis, compared with subjects in the lowest quartile of FLI, the adjusted odds ratios (ORs) in the highest quartile was 2.30 [95% confidence interval

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(CI), 1.36 - 3.90] for increased urinary albumin excretion and 1.93 (95% CI, 1.18 - 3.15) for CKD.

Conclusion: Hepatic steatosis evaluating by FLI is independently associated with increased urinary albumin excretion and prevalence of CKD in middle-aged and elderly Chinese.

Keywords: Fatty liver index; Hepatic steatosis; Increased urinary albumin excretion; Chronic kidney disease

Strengths and Limitations

1. The study was performed in a large population-based cohort in 9,436 Chinese subjects.
2. Findings of the study may be applied to the majority of patients in general practice with suspected hepatic steatosis.
3. Results should be interpreted cautiously due to the observational design of the current study.

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Introduction

As directly affects the global burden of cardiovascular disease mortality, chronic kidney disease (CKD) has become one of the leading public health problem world-wide [1]. Recent national survey conducted between 2007 and 2010 reported that the prevalence of CKD was 10.8%, representing an estimated 119.5 million patients in China with chronic kidney damage [2]. In addition to CKD, an increasing number of studies have provided substantial evidence of albuminuria as a risk factor for future cardiovascular events [3]. Both renal and cardiovascular diseases sharing similar traditional risk factors, such as lipid metabolism disorder, which could have particularly broad implications for the outcome of cardiovascular morbidity and mortality.

Association of hepatic steatosis with CKD development and its impact on the reduction of the estimated glomerular filtration rate (eGFR) have been extensively investigated over the past decade [4]. The substantial evidence linked hepatic steatosis to the increased risk and severity of CKD, which may be a target for the prevention and treatment of the disease [5]. As a convenient scoring system for the presence of hepatic lipid deposits, the fatty liver index (FLI) is a surrogate steatosis biomarker developed in a cohort of patients from the general population [6]. Compared with other techniques for evaluating hepatic steatosis, FLI is simple to obtain as body mass index (BMI), waist circumference (WC), triglycerides (TG) and γ-glutamyltransferase (γ-GGT) are routine measurements in clinical practice. Previous studies have demonstrated that FLI could determine fatty liver disease, incident type 2 diabetes and

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incident hypertension with considerable accuracy [6-8]. Moreover, FLI is associated with insulin resistance early atherosclerosis and risk of coronary heart disease, which could help physicians early detect subjects of greater cardiovascular risk and select patients for intensified lifestyle counseling [9 10].

Clarified the association of FLI with albuminuria and prevalent CKD would probably shed light on the prevention and preemptive treatment of related diseases. Recently, a cross-sectional study was conducted to investigate the association between FLI and CKD by recruiting adults undergoing a health check-up [11]. However, by including only 731 subjects, the study did not evaluate the association between FLI and albuminuria, either. Therefore, we analyzed data from a community-based Chinese population to comprehensively look into the relationship of FLI with both increased urinary albumin excretion and CKD.

Subjects and methods

Study population and design

We performed a cross-sectional study in a community in Guangzhou, China from June to November, 2011. The study population was from the REACTION study and details of this study have been published previously [12-14]. During the recruiting phase, a total of 10,104 residents aged 40 years or older were invited to participate by examination notices or home visits. In total, 9,916 subjects signed the consent form and agreed to participate in the survey, and the participation rate was 98.1%. The subjects who failed to provided information (BMI: n=206; WC: n=62; TG: n=23;

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γ-GGT: n=38; or urinary albumin-to-creatinine ratio [ACR]: n=149) were excluded from the analyses. Accordingly, a total of 9,438 eligible individuals were included in the final data analyses. The study protocol was approved by the Institutional Review Board of the Sun Yat-sen Memorial Hospital affiliated to Sun Yat-sen University and was in accordance with the principle of the Helsinki Declaration II. Written informed consent was obtained from each participant before data collection.

Clinical and biochemical measurements

We collected information on lifestyle factors, sociodemographic characteristics and family history by using a standard questionnaire. Smoking or drinking habit was classified as 'never', 'current' (smoking or drinking regularly in the past 6 months) or 'ever' (cessation of smoking or drinking more than 6 months) [15]. A short form of the International Physical Activity Questionnaire (IPAQ) was used to estimate physical activity at leisure time by adding questions on frequency and duration of moderate or vigorous activities and walking [16]. Separate metabolic equivalent hours per week (MET-h/week) were calculated for evaluation of total physical activity.

All participants completed anthropometrical measurements with the assistance of trained staff by using standard protocols. Three times consecutively blood pressure measurements by the same observer with a 5-minute interval were obtained by an automated electronic device (OMRON, Omron Company, China). The average of three measurements of blood pressure was used for analysis. Body height and body weight were recorded to the nearest 0.1 cm and 0.1 kg while participants were wearing light indoor clothing without shoes. BMI was calculated as weight in

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kilograms divided by height in meters squared (kg/m^2). Obesity was defined as BMI equal or greater than 28 and overweight was defined as BMI equal or greater than 24 and less than 28 [17]. WC was measured at the umbilical level with participant in standing position, at the end of gentle expiration.

Venous blood samples were collected for laboratory tests after an overnight fasting of at least 10 hours. Measurement of fasting plasma glucose (FPG), fasting serum insulin, TG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine, γ-GGT, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was done using an autoanalyser (Beckman CX-7 Biochemical Autoanalyser, Brea, CA, USA).

As surrogate marker of hepatic steatosis, FLI was analyzed based on BMI, WC, TG, and γ-GGT, which has been validated against liver ultrasound in the general population and has been proven accurate in detecting fatty liver [6 10]. FLI is calculated as: $FLI = (e^{0.953 * \log e(TG) + 0.139 * BMI + 0.718 * \log e(GGT) + 0.053 * WC - 15.745}) / (1 + e^{0.953 * \log e (TG) + 0.139 * BMI + 0.718 * \log e (GGT) + 0.053 * WC - 15.745}) * 100$. The abbreviated Modification of Diet in Renal Disease (MDRD) formula recalibrated for Chinese population was used to calculate estimated glomerular filtration rate (GFR) expressed in mL/min per 1.73 m^2 using a formula of $eGFR = 175 \times [\text{serum creatinine} \times 0.011]^{-1.234} \times [\text{age}]^{-0.179} \times [0.79 \text{ if female}]$, where serum creatinine was expressed as μmol/L [18]. Diabetes was diagnosed according to the 1999 World Health Organization diagnostic criteria [19].

Definition of increased urinary albumin excretion, chronic kidney disease and

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non-alcoholic fatty liver disease (NAFLD)

Definitions of abnormalities in albumin excretion were according to the latest guidelines of American Diabetes Association's Standards of Medical Care [20]. The first morning spot urine samples were collected for assessing the ACR. Urine albumin and creatinine were measured by chemiluminescence immunoassay (Siemens Immulite 2000, United States) and the Jaffe' s kinetic method (Biobase-Crystal, Jinan, China) on the automatic analyzer, respectively. ACR was calculated by dividing the urinary albumin concentrations by the urinary creatinine concentrations and expressed in mg/g. The primary and secondary outcome measures were increased urinary albumin excretion and chronic kidney disease (CKD), respectively. Increased urinary albumin excretion was defined according to the ACR ranges greater or equal than 30 mg/g. Chronic kidney disease (CKD) was defined as eGFR less than 60 mL/min per 1.73 m^2 or presence of albuminuria (ACR greater or equal than 30 mg/g). The optimal cutoff value of FLI for predicting NAFLD was 30 in Asian populations [21]. Therefore, we classified the study population in non-current drinking group into NAFLD group (FLI $\geq$ 30) and non-NAFLD group (FLI < 30).

Statistical analysis

Statistical analysis was performed using SAS version 9.2 (SAS Institute Inc, Cary, NC, USA). Continuous variables were presented as means ± standard deviation (SD) except for skewed variables, which were presented as medians (interquartile ranges). Categorical variables were expressed as numbers (proportions). FLI, FPG, TG, ALT,

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AST, γ-GGT and MET-h/week were logarithmically transformed before analysis due to a non-normal distribution. FLI was presented as quartiles and linear regression analysis was used to test for trend across groups. Differences among groups were tested by one-way ANOVA and *post hoc* comparisons were performed by using Bonferroni correction. Comparisons between categorical variables were performed with the χ^2 test.

Pearson's correlations were performed to test the correlations between FLI and the risk factors for kidney disease. Variables significant at $P < 0.20$ in Pearson's correlations were put into the multivariate stepwise linear regression models to identify factors that independently associated with FLI. We analyzed the impact of FLI on the prevalence of increased urinary albumin excretion and CKD. The unadjusted and multivariate-adjusted logistic regression analysis was used to assess the risk of prevalent increased urinary albumin excretion and CKD in relation to each quartile increase in FLI level. Variables considered as potential covariates and significant in the stepwise linear regression were put into multivariate-adjusted logistic regression analysis. Model 1 is unadjusted. Model 2 is adjusted for age. Model 3 is adjusted for age, sex, current smoking status, current drinking status, physical activity, systolic blood pressure (SBP), diastolic blood pressure (DBP), LDL-C, fasting insulin, ALT and AST. Model 4 is adjusted for age, sex, BMI, WC, current smoking status, current drinking status, physical activity, systolic blood pressure (SBP), diastolic blood pressure (DBP), TG, LDL-C, fasting insulin, ALT, AST and γ-GGT. Odds ratios (OR) and the corresponding 95% confidence intervals (95% CI)

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were calculated. Relationship of FLI level with albuminuria and CKD were also explored in subgroups stratified by gender (men/women), age (≥ 60/< 60 years), degree of obesity (normal/overweight/obesity), current smoking (yes/no), current drinking (yes/no), hypertension (yes/no) and diabetes (yes/no). Tests for interaction were performed with including simultaneously each strata factor, the quartiles of FLI level and the respective interaction terms (strata factor multiplied by quartiles of FLI level) in the models.

All statistical tests were two-sided, and a P value < 0.05 was considered statistically significant.

Results

Clinical characteristics of the study population

Among the 9,436 enrolled individuals, the mean age was 55.9 ± 8.0 years. The median FLI was 19.1 with interquartile range 8.6 to 37.4. There were 620 (6.6%) subjects categorized as increased urinary albumin excretion and 753 (8.0%) subjects categorized as CKD, respectively. Table 1 shows the clinical and biochemical characteristics of the participants according to FLI quartiles. Participants with higher FLI level had elevated age, BMI, WC, SBP, DBP, TG, TC, LDL-C, FPG, fasting insulin, ALT, AST, γ - GGT and higher proportions of current smokers and current drinkers (all P for trend < 0.0001). Those with higher FLI level also associated with decreased HDL-C and eGFR (all P for trend < 0.0001).

Associations between FLI and metabolic risk factors

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Analysis of Pearson's correlation showed that age, sex, BMI, WC, SBP, DBP, TG, TC, HDL-C, LDL-C, FPG, fasting insulin, ALT, AST, γ-GGT and eGFR were significantly correlated with FLI level. Further multivariate stepwise linear regression showed that age, sex, BMI, WC, SBP, DBP, TG, LDL-C, fasting insulin, ALT, AST and γ-GGT were independent determinants for FLI level (Table 2).

Associations of FLI with increased urinary albumin excretion and CKD

As shown in Figure.1A, from the lowest quartile to the highest quartile of FLI level, the prevalence of increased urinary albumin excretion was 3.64%, 4.83%, 6.23% and 11.57%, respectively (P for trend < 0.0001). Strikingly, the prevalence of CKD also tended to increase with the elevated FLI quartile (Figure.1B, P for trend < 0.0001). As shown in Table 3, compared with participants in quartile 1 of FLI, univariate logistic regression analysis showed that participants in quartile 2, quartile 3 and quartile 4, respectively, have a significant correlation with increased odds of increased urinary albumin excretion and CKD (all P for trend < 0.0001). In multivariate logistic regression analyses (Model 3), the ORs of increased urinary albumin excretion for increasing FLI quartiles were 1.00 (reference), 0.96 (95% CI 0.66 - 1.39), 1.17 (95% CI 0.77 - 1.77) and 2.30 (95% CI 1.36 - 3.90). Similarly, the ORs of CKD for increasing FLI quartiles in Model 3 were 1.00 (reference), 1.00 (95% CI 0.71 - 1.40), 1.03 (95% CI 0.70 - 1.51) and 1.93 (95% CI 1.18 - 3.15), respectively (Table 3). The prevalence of increased urinary albumin excretion was 51.6% and 29.6% in FLI established NAFLD and non-NAFLD group (P < 0.0001). Similar trends were detected in the prevalence of CKD (NAFLD group: 49.9%; non-NAFLD

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group: 31.5%, P < 0.0001). Compared with participants in the non-NAFLD group, those in NAFLD group had higher prevalence of increased urinary albumin excretion (OR 1.58, 95 % CI 1.18 - 2.13) and CKD (OR 1.39, 95 % CI 1.05 - 1.82) in multivariate logistic regression analyses.

Subgroups analysis of FLI with increased urinary albumin excretion and CKD

As shown in Figure. 2 & 3, the associations of FLI level with increased urinary albumin excretion and CKD were not consistently the same in subgroups analyses. Significant relationship of FLI level with both increased urinary albumin excretion and CKD were detected in women, younger subjects (age less than 60 years), overweight subjects, non-current smokers, non-current drinkers and in those with hypertension or with diabetes (all P < 0.05). In the subgroups analysis, no statistically significance of interaction term between quartiles of FLI and each strata factor was detected.

Discussion

We evaluated the association between hepatic steatosis and kidney disease in a large population of middle-age Chinese subjects from the REACTION study. Presence of fatty liver assessed by FLI was associated with increased urinary albumin excretion and reduction of the eGFR in the present study. The association was independent of potential confounding risk factors. To our current knowledge, this is the largest population-based study to explore the association of FLI with both albuminuria and CKD in Asian population. Early intervention is of great importance

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for albuminuria and CKD, the present findings may just give insights into lipid metabolism for prevention and early detection of the diseases.

Prevalence of obesity was 7.9% (8.4% in males and 7.6% in females) in southern China, which has increased dramatically over the past several decades [22]. The best method for an accurate assessment and diagnosis of hepatic steatosis is histologic analysis of biopsies [23]. However, it is uneconomical to conduct liver biopsies especially by the fact of our large sample population. Hepatic ultrasonic examination is widely used in clinical practice and epidemiological studies in detecting fatty infiltration of the liver [24 25]. However, the noninvasive technique is not sensitive enough to detect mild steatosis and does not allow precise quantification of severity of steatosis in hepatic tissue [26]. As another surrogate marker of histological fatty liver, FLI is defined as the accumulation of excessive liver fat [27]. Based on the former researches, FLI has been proven accurate in detecting fatty liver against liver ultrasound and demonstrating the presence of hepatic fat against magnetic resonance spectroscopy [6 9 10 21]. The superiority of this non-invasive assessment techniques is that a higher score will indicate a higher degree of steatosis in hepatic tissue. However, optimal cut-off point of the FLI for evaluating liver fatty infiltration should be considered as it varied according to the study population [21 28]. Originally, FLI＞60 was suggested to rule in NAFLD in Caucasian subjects. However, the optimal cut-off value of FLI for predicting NAFLD was different in Asian populations. In one recent study, Huang et al. [21] found that FLI could accurately identify NAFLD and the optimal cut-off point was 30 in middle-aged and elderly Chinese. FLI could also

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accurately identify ultrasonography fatty liver in a large scale population in Taiwan but with different optimal cut-off values, while an FLI＞35 for males and＞20 for females rule in NAFLD in their study [28]. Through the results of our research in Chinese subjects, further studies are therefore needed to externally discuss the optimal cut-off point of the FLI for predicting hepatic steatosis.

Detection and prevention of kidney disease progression and urinary albumin excretion is difficult to process in the early stage. Dyslipidemia is increasingly recognized as important pathogenic mechanism in deterioration of renal function. Recently, we conducted a clinical investigation to assess the associations of routine lipid measures with kidney disease in the same cohort. In the study, discordant associations of lipid parameters with renal insufficiency was detected while TG to HDL-C ratio is a better marker for evaluating increased urinary albumin excretion and CKD [29]. As one of the phenotype of dyslipidemia, the pathogeneses of hepatic steatosis is closely related to kidney disease with regard to insulin resistance and chronic inflammation [30]. Hepatokines, which are proteins secreted by hepatocytes, have been found to link to the induction of metabolic phenotypes through inter-organ communication based on recent studies [31]. Because of the high prevalence and burden of the fatty liver disease, it is important to identify which patients are most likely to be exposed to early stage renal injury [32]. Consequently, we closely monitor the association of the hepatic steatosis predict by FLI with prevalent increased urinary albumin excretion and CKD.

Consistent with our findings, a previous study reported that hepatic steatosis

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evaluated by FLI might contribute to CKD development [11]. Elevated albuminuria is well known to be associated with increased risk for early diabetes renal damage, however, the identification and classification of kidney disease was assessed only by eGFR in that study. Moreover, 731 adults that underwent routine health evaluations were included in that study and the small sample size cannot better represent the whole population. By totally including 9,438 subjects and adopting both albuminuria and eGFR for renal damage assessment, data in our study demonstrated that the FLI is associated with kidney disease, which might be an efficient screening indicator for the early prevention of related diseases in Chinese subjects. Recently, an interesting study by Giorda C et al. [33] reported that NAFLD is a dynamic condition in type 2 diabetes subjects and about 5% Italian diabetic patients entering or leaving FLI assessed NAFLD status every year. They found that male sex and established organ damage, especially kidney function, were independent risk predictors for the dynamic NAFLD condition in a longitudinal 3-year analysis. As the similarity in traditional risk factors for both NAFLD and CKD, relationship between the prevalence of earlier stages of kidney damage and the incidence of NAFLD is complex. Longitudinal observation of our cohort are needed to be carried out to determine whether such dynamic condition existed in the Chinese, especially in those with type 2 diabetes.

Alcohol consumption can profoundly disturb the lipid metabolism which have prominent effects on the hepatic tissue steatosis and insulin sensitivity [34]. However, potential health effects regarding alcohol consumption in this field is also worth attaching attention. A meta-analysis of intervention studies by Schrieks et al [35].

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showed that moderate alcohol intake could improve insulin sensitivity by decreasing fasting insulin level in women. Recently, a prospective cohort study found that alcohol consumption was consistently inversely associated with urinary albumin excretion and the risk of developing CKD [36]. Therefore, advice concerning alcohol consumption to subjects with low-grade hepatic tissue steatosis should consider the full range of benefits and risks, especially among those who drink moderately.

Some limitations of the study must be noted. Firstly, owing to the observational design of the current study, we should cautiously interpret the present findings as no causal inference can be drawn. Further prospective studies are therefore needed to determine the precise relationship between FLI and risk of renal diseases. Secondly, by including only Chinese subjects, the results of the present study might not be representative of other ethnic groups, especially for those in the developed or undeveloped countries. To some extent, however, the present study of Chinese population was still a convenience sample and selection bias is inevitable. Thirdly, when evaluating the findings of the present study, the results should be interpreted cautiously due to possible bias from using the indirect indicator FLI to assess fatty liver disease. Moreover, the internal accuracy of FLI for evaluation hepatic steatosis should also be validated by using other techniques, before it can be employed for these purposes. Fourthly, we observed that FLI seem to play a different efficiency for kidney disease assessment in different stratifications. A significant association of FLI with increased urinary albumin excretion and CKD only detected in subjects without current alcohol consumption. Average daily alcohol intake influences the FLI and

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missing such data in the present study doesn't permit comparisons between and within alcoholic and nonalcoholic fatty liver disease groups. To better discriminate alcoholic fatty liver disease and non-alcoholic fatty liver disease, further studies need to clearly described the precise exposure of alcohol use by collecting histories of alcohol intake in a quantitative manner. Fifthly, viral hepatitis infection is one of the most serious infectious diseases worldwide, which can be associated with both liver and kidney disease. Recent survey data showed that the hepatitis B surface antigen and anti-hepatitis C virus–positive rates were already 6.1% and 3.0% in China. Epidemiology of viral hepatitis infection by hepatitis B virus (HBV) and hepatitis C virus (HCV) serological testing, therefore, should be also be evaluate to strength the findings of the present study [37]. Sixthly, although a spectrum of covariates was included in the adjustment, other potential mediators such as daily energy and protein intake and medicine that influence the renin-angiotensin-system of the subjects, should also be considered in the present study.

In conclusion, by including a large population based cohort, the present study provides evidence that increased FLI is independently associated with prevalence of albuminuria and CKD. Further prospective studies are necessary to verify our findings in external populations.

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Figure legends

Figure. 1 Prevalence of increased urinary albumin excretion and CKD in different quartiles of FLI levels. (A) Increased urinary albumin excretion. (B) CKD.

Figure. 2 Risk of prevalent increased urinary albumin excretion with each quartile increase of FLI levels in different subgroups.

Figure. 3 Risk of prevalent CKD with each quartile increase of FLI levels in different subgroups.

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424

425

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Table 1. Characteristics of study population by FLI quartiles

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
n (%)	2360 (25.01)	2359 (24.99)	2359 (24.99)	2360 (25.01)	
Fatty liver index	5.00 (3.27 – 6.74)	13.23 (10.76 – 15.99)	26.96 (22.74 – 31.75)	54.71 (45.40 – 68.10)	
Urinary albumin to creatinine ratio (mg/g)	7.65 (5.59 – 11.12)	8.01 (5.64 – 11.71)	8.06 (5.73 – 11.83)*	8.93 (5.96 – 15.01)*#&	< 0.0001
Age (years)	54.3 ± 7.8	55.8 ± 7.9*	56.5 ± 7.9*#	56.9 ± 8.3*#	< 0.0001
Male [n (%)]	427 (18.09)	593 (25.17)	701 (29.72)	975 (41.31)	< 0.0001
BMI (kg/m^2)	20.6 ± 2.0	22.9 ± 2.0*&	24.4 ± 2.1*#	26.8 ± 3.5*#&	< 0.0001
WC (cm)	72.0 ± 5.8	79.3 ± 5.4*&	84.1 ± 5.5*#	91.3 ± 8.5*#&	< 0.0001
SBP (mmHg)	118.6 ± 14.7	124.5 ± 15.9*&	128.4 ± 15.8*#	132.5 ± 16.1*#&	< 0.0001
DBP (mmHg)	71.2 ± 9.1	74.2 ± 9.3*&	76.5 ± 9.4*#	79.3 ± 9.8*#&	< 0.0001
Current smoking [n (%)]	169 (7.3)	202 (8.7)	227 (9.8)	335 (14.4)	< 0.0001
Current drinking [n (%)]	57 (2.5)	70 (3.0)	68 (2.9)	117 (5.1)	< 0.0001
TG (mmol/L)	0.85 (0.69 – 1.07)	1.12 (0.90 – 1.43) *&	1.49 (1.13 – 1.94) *#	2.10 (1.56 – 3.01) *#&	< 0.0001
TC (mmol/L)	4.79 ± 1.24	5.16 ± 1.22*&	5.35 ± 1.13 *#	5.54 ± 1.17*#&	< 0.0001
HDL-C (mmol/L)	1.45 ± 0.41	1.37 ± 0.35*&	1.29 ± 0.31*#	1.19 ± 0.28*#&	< 0.0001

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LDL-C (mmol/L)	2.82 ± 0.90	3.19 ± 0.94$^{*\&}$	3.31 ± 0.91$^{*\#}$	3.28 ± 0.95$^{*\#}$	< 0.0001
FPG (mmol/L)	5.23 (4.89 – 5.61)	5.33 (4.95 – 5.80) $^{*\&}$	5.47 (5.05 – 5.96) $^{*\#}$	5.73 (5.23 – 6.42) $^{*\#\&}$	< 0.0001
Fasting insulin (μIU/ml)	5.10 (3.90 – 6.50)	6.50 (5.00 – 8.40) $^{*\&}$	7.90 (6.10 – 10.30) $^{*\#}$	10.50 (7.80 – 13.70) $^{*\#\&}$	< 0.0001
ALT (U/L)	10.0 (8.0 – 14.0)	12.0 (9.0 – 16.0) $^{*\&}$	13.0 (10.0 – 17.0) $^{*\#}$	17.0 (12.0 – 24.0) $^{*\#\&}$	< 0.0001
AST (U/L)	17.0 (14.0 – 20.0)	18.0 (15.0 – 21.0) $^{*\&}$	18.0 (15.0 – 22.0) $^{*\#}$	20.0 (17.0 – 25.0) $^{*\#\&}$	< 0.0001
γ-GGT (U/L)	14.0 (11.0 – 17.0)	18.0 (14.0 – 23.0) $^{*\&}$	22.0 (17.0 – 29.0) $^{*\#}$	31.0 (23.0 – 47.0) $^{*\#\&}$	< 0.0001
Serum creatinine (μmol/L)	65.3 ± 15.5	68.8 ± 16.0$^{*\&}$	70.5 ± 16.0$^{*\#}$	74.9 ± 17.2 $^{*\#\&}$	< 0.0001
eGFR (ml/min per 1.73 m^2)	108.0 ± 25.4	102.5 ± 23.7$^{*\&}$	99.9 ± 19.6 $^{*\#}$	95.5 ± 19.5$^{**\#\&}$	< 0.0001
Physical activity (MET-h/week)	24.0 (10.5 – 49.0)	24.0 (10.5 – 45.0)	23.0 (10.5 – 42.0)	21.0 (10.5 – 42.0)*	0.006

1. Data were means ± SD or medians (interquartile ranges) for skewed variables or numbers (proportions) for categorical variables.
2. P for trend was calculated for the linear regression analysis tests across the groups. P values were for the ANOVA or χ^2 analyses across the groups.
3. *P < 0.05 compared with Quartile 1 of fatty liver index; $^{\#}$P < 0.05 compared with Quartile 2 of fatty liver index; $^{\&}$P < 0.05 compared with Quartile 3 of fatty liver index.
4. BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GGT, γ-glutamyltransferase; eGFR, estimated glomerular filtration rate.

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Table 2. Pearson's correlation and stepwise regression analysis of determinants of FLI

	r	P value	Standardized β	P value
Age (years)	0.12	< 0.0001	0.01	0.010
Sex (men=1, women=2)	-0.19	< 0.0001	-0.04	< 0.0001
BMI (kg/m^2)	0.71	< 0.0001	0.30	< 0.0001
WC (cm)	0.78	< 0.0001	0.42	< 0.0001
Physical activity (MET-h/week)	-0.02	0.060	-	-
SBP (mmHg)	0.32	< 0.0001	0.01	0.006
DBP (mmHg)	0.32	< 0.0001	0.01	0.047
TG (mmol/L)	0.68	< 0.0001	0.42	< 0.0001
HDL-C (mmol/L)	-0.26	< 0.0001	-	-
LDL-C (mmol/L)	0.21	< 0.0001	0.06	< 0.0001
FPG (mmol/L)	0.22	< 0.0001	-	-
Fasting insulin (μIU/ml)	0.40	< 0.0001	0.01	0.0002
ALT (U/L)	0.20	< 0.0001	0.05	< 0.0001
AST (U/L)	0.15	< 0.0001	-0.03	< 0.0001
γ-GGT (U/L)	0.35	< 0.0001	0.16	< 0.0001
eGFR (ml/min per 1.73 m^2)	-0.19	< 0.0001	-	-

r, correlation coefficient; β, regression coefficient.

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Table 3. The risk of prevalent albuminuria and CKD according to quartiles of FLI

		Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Increased urinary albumin excretion	Model 1	1	1.34 (1.01 – 1.79)	1.76 (1.34 – 2.31)	3.46 (2.70 – 4.44)	< 0.0001
	Model 2	1	1.29 (0.97 – 1.72)	1.66 (1.27 – 2.19)	3.25 (2.53 – 4.17)	< 0.0001
	Model 3	1	0.94 (0.66 – 1.33)	1.13 (0.81 – 1.59)	2.22 (1.60 – 3.07)	< 0.0001
	Model 4	1	0.96 (0.66 – 1.39)	1.17 (0.77 – 1.77)	2.30 (1.36 – 3.90)	0.001
CKD	Model 1	1	1.47 (1.13 – 1.90)	1.79 (1.39 – 2.30)	3.49 (2.77 – 4.39)	< 0.0001
	Model 2	1	1.39 (1.07 – 1.80)	1.65 (1.28 – 2.12)	3.16 (2.51 – 3.99)	< 0.0001
	Model 3	1	0.99 (0.73 – 1.36)	1.03 (0.75 – 1.40)	1.95 (1.44 – 2.64)	< 0.0001
	Model 4	1	1.00 (0.71 – 1.40)	1.03 (0.70 – 1.51)	1.93 (1.18 – 3.15)	0.012

Data are odds ratios (95% confidence interval). Participants without increased urinary albumin excretion or CKD are defined as 0 and with increased urinary albumin excretion or CKD as 1.

Model 1 is unadjusted.

Model 2 is adjusted for age.

Model 3 is adjusted for age, sex, current smoking status, current drinking status, physical activity, SBP, DBP, LDL-C, fasting insulin, ALT and AST.

Model 4 is adjusted for age, sex, BMI, WC, current smoking status, current drinking status, physical activity, SBP, DBP, TG, LDL-C, fasting insulin, ALT,

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AST and γ-GGT.

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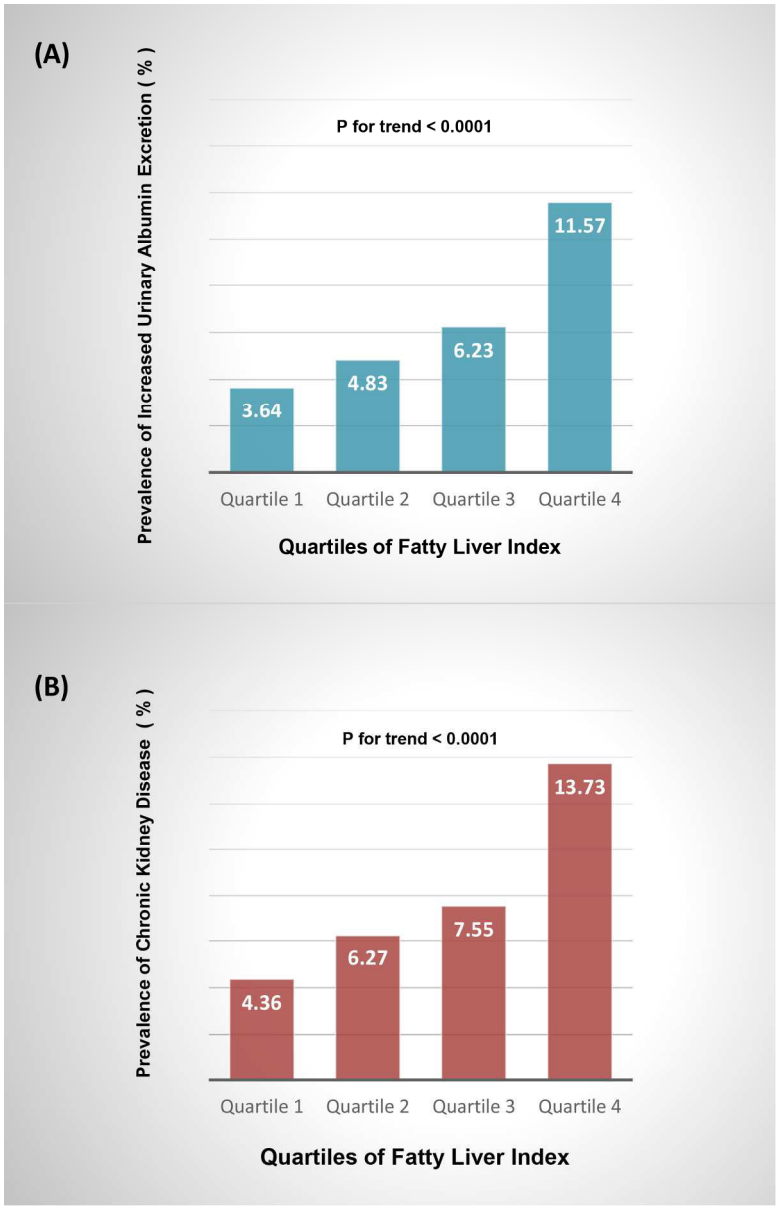

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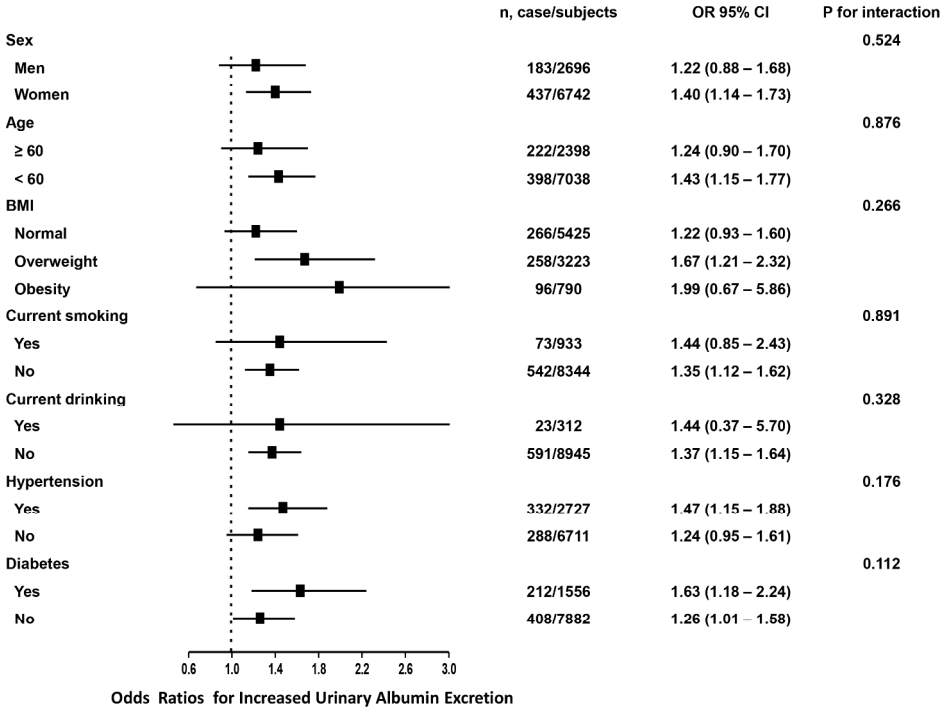

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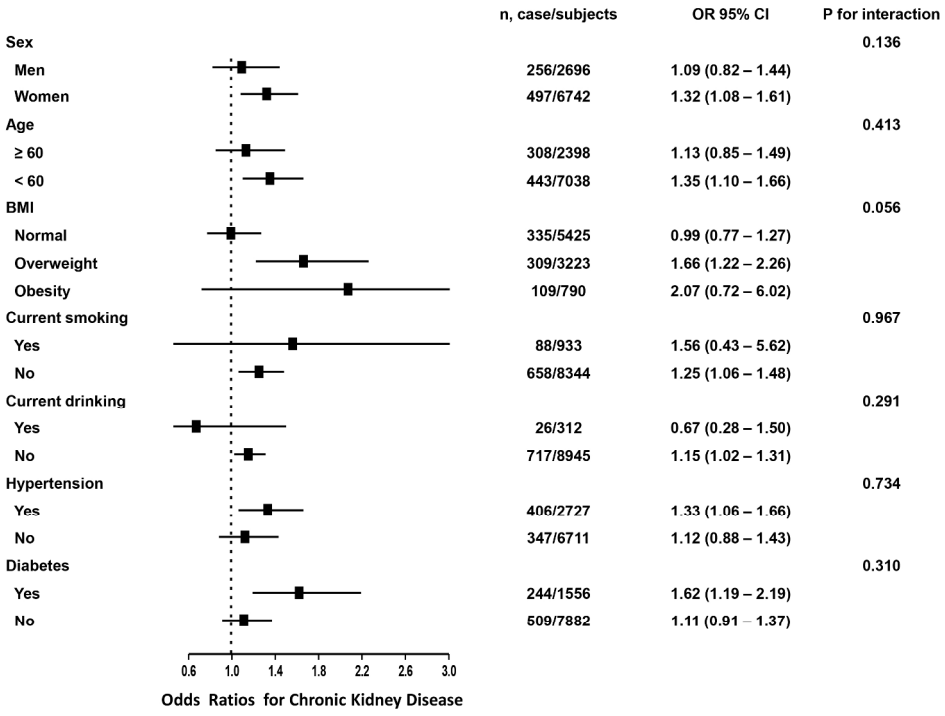

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	**L 1,2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	**L 67-91**
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	**L 105-137**
Objectives	3	State specific objectives, including any prespecified hypotheses	**L 130-137**
Methods			
Study design	4	Present key elements of study design early in the paper	**L 141-146**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	**L 141-179**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	**L 141-153**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	**L 155-207**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	**L 193-207**
Bias	9	Describe any efforts to address potential sources of bias	**L 227-242**
Study size	10	Explain how the study size was arrived at	**L 143-146**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	**L 210-219**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	**L 210-242**
		(*b*) Describe any methods used to examine subgroups and interactions	**L 236-239**
		(*c*) Explain how missing data were addressed	**N.A.**
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	**N.A.**
		(*e*) Describe any sensitivity analyses	**N.A.**
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	**L 248-256**
		(b) Give reasons for non-participation at each stage	**N.A.**
		(c) Consider use of a flow diagram	**N.A.**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	**L 248-262**
		(b) Indicate number of participants with missing data for each variable of	**L 248-**

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		interest	**292**
Outcome data	15*	Report numbers of outcome events or summary measures	**L 264-283**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	**L 264-283**
		(*b*) Report category boundaries when continuous variables were categorized	**L 210-219**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	**N.A.**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**L 285-292**
Discussion			
Key results	18	Summarise key results with reference to study objectives	**L 295-303**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**L 374-403**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**L 329-373**
Generalisability	21	Discuss the generalisability (external validity) of the study results	**L 377-381**
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	**L 47-59**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Fatty liver index is associated with albuminuria and chronic kidney disease: a population-based study

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All authors believe that the manuscript represents valid work and have reviewed and approved the final version. The work has not been published previously, and not under consideration for publication elsewhere, in part or in whole.

The author contribution lists

Conceived and designed the experiments: Y. L. and K. S.

Performed the experiments: F. L., Y. Q., W. F., C. C., K. S. and D. L.

Analyzed the data: K. S. and M. R.

Wrote the manuscript: K.S. and D. L.

Data Sharing Statement

The work described was original research that has not been published previously, and not under consideration for publication elsewhere, in part or in whole. All authors believe that the manuscript represents valid work and have reviewed and approved the final version. Main document data and additional unpublished data from the study are available by sending Email to lizyhenu@163.com with proper purposes.

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Conflict of interests

The authors have declared that no competing interests exist.

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ABSTRACT

Objectives: The effects of lipid metabolism disorder on the renal damage have drawn much attention. By using the fatty liver index (FLI) as a validated indicator of hepatic steatosis, this study aims at provide insight about the possible links between fatty liver and development of chronic kidney disease (CKD).

Setting: hospital.

Participants: We performed a population-based study in 9,436 subjects aged 40 years or older.

Primary and secondary outcome measures: FLI is calculated by using an algorithm based on body mass index (BMI), waist circumference (WC), triglycerides (TG) and γ-glutamyltransferase (γ-GGT). Increased urinary albumin excretion was defined according to the urinary albumin-to-creatinine ratio ranges greater or equal than 30 mg/g. CKD was defined as estimated glomerular filtration rate (eGFR) less than 60 mL/min per 1.73 m² or presence of albuminuria.

Results: There were 620 (6.6%) subjects categorized as increased urinary albumin excretion and 753 (8.0%) subjects categorized as CKD. Participants with higher FLI had increased age, blood pressure, low-density lipoprotein cholesterol, fasting plasma glucose, fasting insulin and decreased eGFR level. Prevalence of increased urinary albumin excretion and CKD tended to increase with the elevated FLI quartiles. In logistic regression analysis, compared with subjects in the lowest quartile of FLI, the adjusted odds ratios (ORs) in the highest quartile was 2.30 [95% confidence interval

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(CI), 1.36 - 3.90] for increased urinary albumin excretion and 1.93 (95% CI, 1.18 - 3.15) for CKD.

Conclusion: Hepatic steatosis evaluating by FLI is independently associated with increased urinary albumin excretion and prevalence of CKD in middle-aged and elderly Chinese.

Keywords: Fatty liver index; Hepatic steatosis; Increased urinary albumin excretion; Chronic kidney disease

Strengths and Limitations

1. The study was performed in a large population-based cohort in 9,436 Chinese subjects.
2. Findings of the study may be applied to the majority of patients in general practice with suspected hepatic steatosis.
3. Results should be interpreted cautiously due to the observational design of the current study.

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Introduction

Chronic kidney disease (CKD) has become one of the leading public health problem world-wide [1]. Recent national survey conducted between 2007 and 2010 reports that the prevalence of CKD was 10.8%, representing an estimated 119.5 million patients in China are with chronic kidney damage [2]. In addition to CKD, an increasing number of studies have provided substantial evidence of albuminuria as a risk factor for future cardiovascular events [3]. Both renal and cardiovascular diseases sharing similar traditional risk factors, such as lipid metabolism disorder, could have particularly broad implications for the outcome of cardiovascular morbidity and mortality.

Association of hepatic steatosis with CKD development and its impact on the reduction of the estimated glomerular filtration rate (eGFR) have been extensively investigated over the past decade [4]. The substantial evidence linked hepatic steatosis to the increased risk and severity of CKD, which may be a target for the prevention and treatment of the disease [5]. As a convenient scoring system for the presence of hepatic lipid deposits, the fatty liver index (FLI) is a surrogate steatosis biomarker developed in a cohort of patients from the general population [6]. Compared with other techniques for evaluating hepatic steatosis, FLI is simple to obtain as body mass index (BMI), waist circumference (WC), triglycerides (TG) and γ-glutamyltransferase (γ-GGT) are routine measurements in clinical practice. Previous studies have demonstrated that FLI could determine fatty liver disease, incident type 2 diabetes and incident hypertension with considerable accuracy [6-8]. Moreover, FLI is associated with

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insulin resistance early atherosclerosis and risk of coronary heart disease, which could help physicians early detect subjects of greater cardiovascular risk and select patients for intensified lifestyle counseling [9 10].

Clarifying the association of FLI with albuminuria and prevalent CKD would probably shed light on the prevention and preemptive treatment of related diseases. Recently, a cross-sectional study was conducted to investigate the association between FLI and CKD by recruiting adults undergoing a health check-up [11]. However, by including only 731 subjects, the study did not evaluate the association between FLI and albuminuria, either. Therefore, we analyzed data from a community-based Chinese population to comprehensively look into the relationship of FLI with both increased urinary albumin excretion and CKD.

Subjects and methods

Study population and design

We performed a cross-sectional study in a community in Guangzhou, China from June to November, 2011. The study population was from the REACTION study and details of this study have been published previously [12-14]. During the recruiting phase, a total of 10,104 residents aged 40 years or older were invited to participate by examination notices or home visits. In total, 9,916 subjects signed the consent form and agreed to participate in the survey. The participation rate was 98.1%. The subjects who failed to provide information (BMI: n=206; WC: n=62; TG: n=23; γ-GGT: n=38; or urinary albumin-to-creatinine ratio [ACR]: n=149) were excluded from the

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analyses. Accordingly, a total of 9,438 eligible individuals were included in the final data analyses. The study protocol was approved by the Institutional Review Board of the Sun Yat-sen Memorial Hospital affiliated to Sun Yat-sen University and was in accordance with the principle of the Helsinki Declaration II. Written informed consent was obtained from each participant before data collection.

Clinical and biochemical measurements

We collected information on lifestyle factors, sociodemographic characteristics and family history by using a standard questionnaire. Smoking or drinking habit was classified as 'never', 'current' (smoking or drinking regularly in the past 6 months) or 'ever' (cessation of smoking or drinking more than 6 months) [15]. A short form of the International Physical Activity Questionnaire (IPAQ) was used to estimate physical activity at leisure time by adding questions on frequency and duration of moderate or vigorous activities and walking [16]. Separate metabolic equivalent hours per week (MET-h/week) were calculated for evaluation of total physical activity.

All participants completed anthropometrical measurements are with the assistance of trained staff by using standard protocols. Three times consecutively blood pressure measurements by the same observer in a 5-minute interval were obtained by an automated electronic device (OMRON, Omron Company, China). The average of three measurements of blood pressure was used for analysis. Body height and body weight were recorded to the nearest 0.1 cm and 0.1 kg while participants were wearing light indoor clothing without shoes. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Obesity was defined as BMI

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equal or greater than 28 and overweight was defined as BMI equal or greater than 24 and less than 28 [17]. WC was measured at the umbilical level with participant in standing position, at the end of gentle expiration.

Venous blood samples were collected for laboratory tests after an overnight fasting of at least 10 hours. Measurement of fasting plasma glucose (FPG), fasting serum insulin, TG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine, γ-GGT, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was done using an autoanalyser (Beckman CX-7 Biochemical Autoanalyser, Brea, CA, USA).

As surrogate marker of hepatic steatosis, FLI was analyzed based on BMI, WC, TG, and γ-GGT, which has been validated against liver ultrasound in the general population and has been proven accurate in detecting fatty liver [6 10]. FLI is calculated as: $FLI = (e^{0.953 * \log e(TG) + 0.139 * BMI + 0.718 * \log e(GGT) + 0.053 * WC - 15.745}) / (1 + e^{0.953 * \log e (TG) +0.139 * BMI + 0.718 * \log e (GGT) + 0.053 * WC - 15.745}) * 100$. The abbreviated Modification of Diet in Renal Disease (MDRD) formula recalibrated for Chinese population was used to calculate estimated glomerular filtration rate (GFR) expressed in mL/min per 1.73 m^2 using a formula of $eGFR = 175 \times [\text{serum creatinine} \times 0.011]^{-1.234} \times [\text{age}]^{-0.179} \times [0.79 \text{ if female}]$, where serum creatinine was expressed as μmol/L [18]. Diabetes was diagnosed according to the 1999 World Health Organization diagnostic criteria [19].

Definition of increased urinary albumin excretion, chronic kidney disease and non-alcoholic fatty liver disease (NAFLD)

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Definitions of abnormalities in albumin excretion were according to the latest guidelines of American Diabetes Association's Standards of Medical Care [20]. The first morning spot urine samples were collected for assessing the ACR. Urine albumin and creatinine were measured by chemiluminescence immunoassay (Siemens Immulite 2000, United States) and the Jaffe' s kinetic method (Biobase-Crystal, Jinan, China) on the automatic analyzer, respectively. ACR was calculated by dividing the urinary albumin concentrations by the urinary creatinine concentrations and expressed in mg/g. The primary and secondary outcome measures were increased urinary albumin excretion and chronic kidney disease (CKD), respectively. Increased urinary albumin excretion was defined according to the ACR ranges greater or equal than 30 mg/g. Chronic kidney disease (CKD) was defined as eGFR less than 60 mL/min per 1.73 m^2 or presence of albuminuria (ACR greater or equal than 30 mg/g). The optimal cutoff value of FLI for predicting NAFLD was 30 in Asian populations [21]. Therefore, we classified the study population in non-current drinking group into NAFLD group (FLI $\geq$ 30) and non-NAFLD group (FLI < 30).

Statistical analysis

Statistical analysis was performed using SAS version 9.2 (SAS Institute Inc, Cary, NC, USA). Continuous variables were presented as means ± standard deviation (SD) except for skewed variables, which were presented as medians (interquartile ranges). Categorical variables were expressed as numbers (proportions). FLI, FPG, TG, ALT, AST, γ-GGT and MET-h/week were logarithmically transformed before analysis due

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to a non-normal distribution. FLI was presented as quartiles and linear regression analysis was used to test for trend across groups. Differences among groups were tested by one-way ANOVA and *post hoc* comparisons were performed by using Bonferroni correction. Comparisons between categorical variables were performed with the χ^2 test.

Pearson's correlations were performed to test the correlations between FLI and the risk factors for kidney disease. Variables significant at $P < 0.20$ in Pearson's correlations were put into the multivariate stepwise linear regression models to identify factors that independently associated with FLI. We analyzed the impact of FLI on the prevalence of increased urinary albumin excretion and CKD. The unadjusted and multivariate-adjusted logistic regression analysis was used to assess the risk of prevalent increased urinary albumin excretion and CKD in relation to each quartile increase in FLI level. Variables considered as potential covariates and significant in the stepwise linear regression were put into multivariate-adjusted logistic regression analysis. Model 1 is unadjusted. Model 2 is adjusted for age. Model 3 is adjusted for age, sex, current smoking status, current drinking status, physical activity, systolic blood pressure (SBP), diastolic blood pressure (DBP), LDL-C, fasting insulin, ALT and AST. Model 4 is adjusted for age, sex, BMI, WC, current smoking status, current drinking status, physical activity, systolic blood pressure (SBP), diastolic blood pressure (DBP), TG, LDL-C, fasting insulin, ALT, AST and γ-GGT. Odds ratios (OR) and the corresponding 95% confidence intervals (95% CI) were calculated. Relationship of FLI level with albuminuria and CKD were also

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explored in subgroups stratified by gender (men/women), age (≥ 60/< 60 years), degree of obesity (normal/overweight/obesity), current smoking (yes/no), current drinking (yes/no), hypertension (yes/no) and diabetes (yes/no). Tests for interaction were performed with including simultaneously each strata factor, the quartiles of FLI level and the respective interaction terms (strata factor multiplied by quartiles of FLI level) in the models.

All statistical tests were two-sided, and a P value < 0.05 was considered statistically significant.

Results

Clinical characteristics of the study population

Among the 9,436 enrolled individuals, the mean age was 55.9 ± 8.0 years. The median FLI was 19.1 with interquartile range 8.6 to 37.4. There were 620 (6.6%) subjects categorized as increased urinary albumin excretion and 753 (8.0%) subjects categorized as CKD, respectively. Table 1 shows the clinical and biochemical characteristics of the participants according to FLI quartiles. Participants with higher FLI level had elevated age, BMI, WC, SBP, DBP, TG, TC, LDL-C, FPG, fasting insulin, ALT, AST, γ - GGT and higher proportions of current smokers and current drinkers (all P for trend < 0.0001). Those with higher FLI level also associated with decreased HDL-C and eGFR (all P for trend < 0.0001).

Associations between FLI and metabolic risk factors

Analysis of Pearson's correlation showed that age, sex, BMI, WC, SBP, DBP, TG,

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TC, HDL-C, LDL-C, FPG, fasting insulin, ALT, AST, γ-GGT and eGFR were significantly correlated with FLI level. Further multivariate stepwise linear regression showed that age, sex, BMI, WC, SBP, DBP, TG, LDL-C, fasting insulin, ALT, AST and γ-GGT were independent determinants for FLI level (Table 2).

Associations of FLI with increased urinary albumin excretion and CKD

As shown in Figure.1A, from the lowest quartile to the highest quartile of FLI level, the prevalence of increased urinary albumin excretion was 3.64%, 4.83%, 6.23% and 11.57%, respectively (P for trend < 0.0001). Strikingly, the prevalence of CKD also tended to increase with the elevated FLI quartile (Figure.1B, P for trend < 0.0001). As shown in Table 3, compared with participants in quartile 1 of FLI, univariate logistic regression analysis showed that participants in quartile 2, quartile 3 and quartile 4, respectively, have a significant correlation with increased odds of increased urinary albumin excretion and CKD (all P for trend < 0.0001). In multivariate logistic regression analyses (Model 3), the ORs of increased urinary albumin excretion for increasing FLI quartiles were 1.00 (reference), 0.96 (95% CI 0.66 - 1.39), 1.17 (95% CI 0.77 - 1.77) and 2.30 (95% CI 1.36 - 3.90). Similarly, the ORs of CKD for increasing FLI quartiles in Model 3 were 1.00 (reference), 1.00 (95% CI 0.71 - 1.40), 1.03 (95% CI 0.70 - 1.51) and 1.93 (95% CI 1.18 - 3.15), respectively (Table 3). The prevalence of increased urinary albumin excretion was 51.6% and 29.6% in FLI established NAFLD and non-NAFLD group (P < 0.0001). Similar trends were detected in the prevalence of CKD (NAFLD group: 49.9%; non-NAFLD group: 31.5%, P < 0.0001). Compared with participants in the non-NAFLD group,

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those in NAFLD group had higher prevalence of increased urinary albumin excretion (OR 1.58, 95 % CI 1.18 - 2.13) and CKD (OR 1.39, 95 % CI 1.05 - 1.82) in multivariate logistic regression analyses.

Subgroups analysis of FLI with increased urinary albumin excretion and CKD

As shown in Figure. 2 & 3, the associations of FLI level with increased urinary albumin excretion and CKD were not consistently the same in subgroups analyses. Significant relationship of FLI level with both increased urinary albumin excretion and CKD were detected in women, younger subjects (age less than 60 years), overweight subjects, non-current smokers, non-current drinkers and in those with hypertension or with diabetes (all $P < 0.05$). In the subgroups analysis, no statistically significance of interaction term between quartiles of FLI and each strata factor was detected.

Discussion

We evaluated the association between hepatic steatosis and kidney disease in a large population of middle-age Chinese subjects from the REACTION study. Presence of fatty liver assessed by FLI was associated with increased urinary albumin excretion and reduction of the eGFR in the present study. The association was independent of potential confounding risk factors. To our current knowledge, this is the largest population-based study to explore the association of FLI with both albuminuria and CKD in Asian population. Early intervention is of great importance for albuminuria and CKD, the present findings may just give insights into lipid

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metabolism for prevention and early detection of the diseases.

The problem of obesity and NAFLD are now increasingly recognized in the Asian population. Prevalence of obesity was 7.9% (8.4% in males and 7.6% in females) in southern China, which has increased dramatically over the past several decades [22]. There is a strong correlation between established obesity and incidence of NAFLD. Pooled prevalence of NAFLD diagnosed by ultrasound, computed tomography scan and magnetic resonance was estimated to be 27.4% in subjects aged over 30 years from Asian countries [23]. Even among the non-obese Chinese, 8.9% developed NAFLD in five years from 2006 to 2011 [24]. Therefore, early and accurate diagnosis of NAFLD is of great importance. The best method for an accurate assessment and diagnosis of hepatic steatosis is histologic analysis of biopsies [25]. However, it is uneconomical to conduct liver biopsies especially by the fact of our large sample population. Hepatic ultrasonic examination is widely used in clinical practice and epidemiological studies in detecting fatty infiltration of the liver [26 27]. However, the noninvasive technique is not sensitive enough to detect mild steatosis and does not allow precise quantification of severity of steatosis in hepatic tissue [28].

As another surrogate marker of histological fatty liver, FLI is defined as the accumulation of excessive liver fat [29]. Based on the former researches, FLI has been proven accurate in detecting fatty liver against liver ultrasound and demonstrating the presence of hepatic fat against magnetic resonance spectroscopy [6 9 10 21]. The superiority of this non-invasive assessment techniques is that a higher score will indicate a higher degree of steatosis in hepatic tissue. However, optimal cut-off point

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of the FLI for evaluating liver fatty infiltration should be considered as it varied according to the study population [21 30]. Originally, FLI＞60 was suggested to rule in NAFLD in Caucasian subjects. However, the optimal cut-off value of FLI for predicting NAFLD was different in Asian populations. In one recent study, Huang et al. [21] found that FLI could accurately identify NAFLD and the optimal cut-off point was 30 in middle-aged and elderly Chinese. FLI could also accurately identify ultrasonography fatty liver in a large scale population in Taiwan but with different optimal cut-off values, while an FLI＞35 for males and＞20 for females rule in NAFLD in their study [30]. Through the results of our research in Chinese subjects, further studies are therefore needed to externally discuss the optimal cut-off point of the FLI for predicting hepatic steatosis.

Detection and prevention of kidney disease progression and urinary albumin excretion is difficult to process in the early stage. Dyslipidemia is increasingly recognized as important pathogenic mechanism in deterioration of renal function. Recently, we conducted a clinical investigation to assess the associations of routine lipid measures with kidney disease in the same cohort. In the study, discordant associations of lipid parameters with renal insufficiency was detected while TG to HDL-C ratio is a better marker for evaluating increased urinary albumin excretion and CKD [31]. As one of the phenotype of dyslipidemia, the pathogeneses of hepatic steatosis is closely related to kidney disease with regard to insulin resistance and chronic inflammation [32]. Hepatokines, which are proteins secreted by hepatocytes, have been found to link to the induction of metabolic phenotypes through inter-organ

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communication based on recent studies [33]. Because of the high prevalence and burden of the fatty liver disease, it is important to identify which patients are most likely to be exposed to early stage renal injury [23]. Consequently, we closely monitor the association of the hepatic steatosis predict by FLI with prevalent increased urinary albumin excretion and CKD.

Consistent with our findings, a previous study reported that hepatic steatosis evaluated by FLI might contribute to CKD development [11]. Elevated albuminuria is well known to be associated with increased risk for early diabetes renal damage, however, the identification and classification of kidney disease was assessed only by eGFR in that study. Moreover, 731 adults that underwent routine health evaluations were included in that study and the small sample size cannot better represent the whole population. By totally including 9,438 subjects and adopting both albuminuria and eGFR for renal damage assessment, data in our study demonstrated that the FLI is associated with kidney disease, which might be an efficient screening indicator for the early prevention of related diseases in Chinese subjects. Recently, an interesting study by Giorda C et al. [34] reported that NAFLD is a dynamic condition in type 2 diabetes subjects and about 5% Italian diabetic patients entering or leaving FLI assessed NAFLD status every year. They found that male sex and established organ damage, especially kidney function, were independent risk predictors for the dynamic NAFLD condition in a longitudinal 3-year analysis. As the similarity in traditional risk factors for both NAFLD and CKD, relationship between the prevalence of earlier stages of kidney damage and the incidence of NAFLD is complex. Longitudinal observation of

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our cohort are needed to be carried out to determine whether such dynamic condition existed in the Chinese, especially in those with type 2 diabetes.

Alcohol consumption can profoundly disturb the lipid metabolism which have prominent effects on the hepatic tissue steatosis and insulin sensitivity [35]. However, potential health effects regarding alcohol consumption in this field is also worth attaching attention. A meta-analysis of intervention studies by Schrieks et al [36]. showed that moderate alcohol intake could improve insulin sensitivity by decreasing fasting insulin level in women. Recently, a prospective cohort study found that alcohol consumption was consistently inversely associated with urinary albumin excretion and the risk of developing CKD [37]. Therefore, advice concerning alcohol consumption to subjects with low-grade hepatic tissue steatosis should consider the full range of benefits and risks, especially among those who drink moderately.

Some limitations of the study must be noted. Firstly, owing to the observational design of the current study, we should cautiously interpret the present findings as no causal inference can be drawn. Further prospective studies are therefore needed to determine the precise relationship between FLI and risk of renal diseases. Secondly, by including only Chinese subjects, the results of the present study might not be representative of other ethnic groups, especially for those in the developed or undeveloped countries. To some extent, however, the present study of Chinese population was still a convenience sample and selection bias is inevitable. Thirdly, when evaluating the findings of the present study, the results should be interpreted cautiously due to possible bias from using the indirect indicator FLI to assess fatty

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liver disease. The calculated FLI may relate to various liver diseases with associated steatosis and not only NAFLD, despite the fact that metabolic disturbances make obesity related steatosis likely. The internal accuracy of FLI for evaluation hepatic steatosis should also be validated by using other techniques, before it can be employed for these purposes. Fourthly, we observed that FLI seem to play a different efficiency for kidney disease assessment in different stratifications. A significant association of FLI with increased urinary albumin excretion and CKD only detected in subjects without current alcohol consumption. Average daily alcohol intake influences the FLI and missing such data in the present study doesn't permit comparisons between and within alcoholic and nonalcoholic fatty liver disease groups. To better discriminate alcoholic fatty liver disease and non-alcoholic fatty liver disease, further studies need to clearly described the precise exposure of alcohol use by collecting histories of alcohol intake in a quantitative manner. Fifthly, viral hepatitis infection is one of the most serious infectious diseases worldwide, which can be associated with both liver and kidney disease. Recent survey data showed that the hepatitis B surface antigen and anti-hepatitis C virus–positive rates were already 6.1% and 3.0% in China. Epidemiology of viral hepatitis infection by hepatitis B virus (HBV) and hepatitis C virus (HCV) serological testing, therefore, should be also be evaluate to strength the findings of the present study [38]. Sixthly, although a spectrum of covariates was included in the adjustment, other potential mediators such as daily energy and protein intake and medicine that influence the renin-angiotensin-system of the subjects, should also be considered in the present

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study.

In conclusion, by including a large population based cohort, the present study provides evidence that increased FLI is independently associated with prevalence of albuminuria and CKD. Findings of the present study suggested us should pay more attention to albuminuria and eGFR variation in patients with dyslipidemia and fatty liver disease. Further prospective studies are necessary to verify our findings in external populations.

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Figure legends

Figure. 1 Prevalence of increased urinary albumin excretion and CKD in different quartiles of FLI levels. (A) Increased urinary albumin excretion. (B) CKD.

Figure. 2 Risk of prevalent increased urinary albumin excretion with each quartile increase of FLI levels in different subgroups.

Figure. 3 Risk of prevalent CKD with each quartile increase of FLI levels in different subgroups.

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434

435

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Table 1. Characteristics of study population by FLI quartiles

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
n (%)	2360 (25.01)	2359 (24.99)	2359 (24.99)	2360 (25.01)	
Fatty liver index	5.00 (3.27 – 6.74)	13.23 (10.76 – 15.99)	26.96 (22.74 – 31.75)	54.71 (45.40 – 68.10)	
Urinary albumin to creatinine ratio (mg/g)	7.65 (5.59 – 11.12)	8.01 (5.64 – 11.71)	8.06 (5.73 – 11.83)*	8.93 (5.96 – 15.01)*#&	< 0.0001
Age (years)	54.3 ± 7.8	55.8 ± 7.9*	56.5 ± 7.9*#	56.9 ± 8.3*#	< 0.0001
Male [n (%)]	427 (18.09)	593 (25.17)	701 (29.72)	975 (41.31)	< 0.0001
BMI (kg/m^2)	20.6 ± 2.0	22.9 ± 2.0*&	24.4 ± 2.1*#	26.8 ± 3.5*#&	< 0.0001
WC (cm)	72.0 ± 5.8	79.3 ± 5.4*&	84.1 ± 5.5*#	91.3 ± 8.5*#&	< 0.0001
SBP (mmHg)	118.6 ± 14.7	124.5 ± 15.9*&	128.4 ± 15.8*#	132.5 ± 16.1*#&	< 0.0001
DBP (mmHg)	71.2 ± 9.1	74.2 ± 9.3*&	76.5 ± 9.4*#	79.3 ± 9.8*#&	< 0.0001
Current smoking [n (%)]	169 (7.3)	202 (8.7)	227 (9.8)	335 (14.4)	< 0.0001
Current drinking [n (%)]	57 (2.5)	70 (3.0)	68 (2.9)	117 (5.1)	< 0.0001
TG (mmol/L)	0.85 (0.69 – 1.07)	1.12 (0.90 – 1.43) *&	1.49 (1.13 – 1.94) *#	2.10 (1.56 – 3.01) *#&	< 0.0001
TC (mmol/L)	4.79 ± 1.24	5.16 ± 1.22*&	5.35 ± 1.13 *#	5.54 ± 1.17*#&	< 0.0001
HDL-C (mmol/L)	1.45 ± 0.41	1.37 ± 0.35*&	1.29 ± 0.31*#	1.19 ± 0.28*#&	< 0.0001

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LDL-C (mmol/L)	2.82 ± 0.90	3.19 ± 0.94$^{*\&}$	3.31 ± 0.91$^{*\#}$	3.28 ± 0.95$^{*\#}$	< 0.0001
FPG (mmol/L)	5.23 (4.89 – 5.61)	5.33 (4.95 – 5.80) $^{*\&}$	5.47 (5.05 – 5.96) $^{*\#}$	5.73 (5.23 – 6.42) $^{*\#\&}$	< 0.0001
Fasting insulin (μIU/ml)	5.10 (3.90 – 6.50)	6.50 (5.00 – 8.40) $^{*\&}$	7.90 (6.10 – 10.30) $^{*\#}$	10.50 (7.80 – 13.70) $^{*\#\&}$	< 0.0001
ALT (U/L)	10.0 (8.0 – 14.0)	12.0 (9.0 – 16.0) $^{*\&}$	13.0 (10.0 – 17.0) $^{*\#}$	17.0 (12.0 – 24.0) $^{*\#\&}$	< 0.0001
AST (U/L)	17.0 (14.0 – 20.0)	18.0 (15.0 – 21.0) $^{*\&}$	18.0 (15.0 – 22.0) $^{*\#}$	20.0 (17.0 – 25.0) $^{*\#\&}$	< 0.0001
γ-GGT (U/L)	14.0 (11.0 – 17.0)	18.0 (14.0 – 23.0) $^{*\&}$	22.0 (17.0 – 29.0) $^{*\#}$	31.0 (23.0 – 47.0) $^{*\#\&}$	< 0.0001
Serum creatinine (μmol/L)	65.3 ± 15.5	68.8 ± 16.0$^{*\&}$	70.5 ± 16.0$^{*\#}$	74.9 ± 17.2 $^{*\#\&}$	< 0.0001
eGFR (ml/min per 1.73 m^2)	108.0 ± 25.4	102.5 ± 23.7$^{*\&}$	99.9 ± 19.6 $^{*\#}$	95.5 ± 19.5$^{**\#\&}$	< 0.0001
Physical activity (MET-h/week)	24.0 (10.5 – 49.0)	24.0 (10.5 – 45.0)	23.0 (10.5 – 42.0)	21.0 (10.5 – 42.0)*	0.006

1. Data were means ± SD or medians (interquartile ranges) for skewed variables or numbers (proportions) for categorical variables.
2. P for trend was calculated for the linear regression analysis tests across the groups. P values were for the ANOVA or χ^2 analyses across the groups.
3. *P < 0.05 compared with Quartile 1 of fatty liver index; $^{\#}$P < 0.05 compared with Quartile 2 of fatty liver index; $^{\&}$P < 0.05 compared with Quartile 3 of fatty liver index.
4. BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GGT, γ-glutamyltransferase; eGFR, estimated glomerular filtration rate.

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Table 2. Pearson's correlation and stepwise regression analysis of determinants of FLI

	r	P value	Standardized β	P value
Age (years)	0.12	< 0.0001	0.01	0.010
Sex (men=1, women=2)	-0.19	< 0.0001	-0.04	< 0.0001
BMI (kg/m^2)	0.71	< 0.0001	0.30	< 0.0001
WC (cm)	0.78	< 0.0001	0.42	< 0.0001
Physical activity (MET-h/week)	-0.02	0.060	-	-
SBP (mmHg)	0.32	< 0.0001	0.01	0.006
DBP (mmHg)	0.32	< 0.0001	0.01	0.047
TG (mmol/L)	0.68	< 0.0001	0.42	< 0.0001
HDL-C (mmol/L)	-0.26	< 0.0001	-	-
LDL-C (mmol/L)	0.21	< 0.0001	0.06	< 0.0001
FPG (mmol/L)	0.22	< 0.0001	-	-
Fasting insulin (μIU/ml)	0.40	< 0.0001	0.01	0.0002
ALT (U/L)	0.20	< 0.0001	0.05	< 0.0001
AST (U/L)	0.15	< 0.0001	-0.03	< 0.0001
γ-GGT (U/L)	0.35	< 0.0001	0.16	< 0.0001
eGFR (ml/min per 1.73 m^2)	-0.19	< 0.0001	-	-

r, correlation coefficient; β, regression coefficient.

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556

Table 3. The risk of prevalent albuminuria and CKD according to quartiles of FLI

		Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Increased urinary albumin excretion	Model 1	1	1.34 (1.01 – 1.79)	1.76 (1.34 – 2.31)	3.46 (2.70 – 4.44)	< 0.0001
	Model 2	1	1.29 (0.97 – 1.72)	1.66 (1.27 – 2.19)	3.25 (2.53 – 4.17)	< 0.0001
	Model 3	1	0.94 (0.66 – 1.33)	1.13 (0.81 – 1.59)	2.22 (1.60 – 3.07)	< 0.0001
	Model 4	1	0.96 (0.66 – 1.39)	1.17 (0.77 – 1.77)	2.30 (1.36 – 3.90)	0.001
CKD	Model 1	1	1.47 (1.13 – 1.90)	1.79 (1.39 – 2.30)	3.49 (2.77 – 4.39)	< 0.0001
	Model 2	1	1.39 (1.07 – 1.80)	1.65 (1.28 – 2.12)	3.16 (2.51 – 3.99)	< 0.0001
	Model 3	1	0.99 (0.73 – 1.36)	1.03 (0.75 – 1.40)	1.95 (1.44 – 2.64)	< 0.0001
	Model 4	1	1.00 (0.71 – 1.40)	1.03 (0.70 – 1.51)	1.93 (1.18 – 3.15)	0.012

Data are odds ratios (95% confidence interval). Participants without increased urinary albumin excretion or CKD are defined as 0 and with increased urinary albumin excretion or CKD as 1.
Model 1 is unadjusted.
Model 2 is adjusted for age.
Model 3 is adjusted for age, sex, current smoking status, current drinking status, physical activity, SBP, DBP, LDL-C, fasting insulin, ALT and AST.
Model 4 is adjusted for age, sex, BMI, WC, current smoking status, current drinking status, physical activity, SBP, DBP, TG, LDL-C, fasting insulin, ALT,

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AST and γ-GGT.

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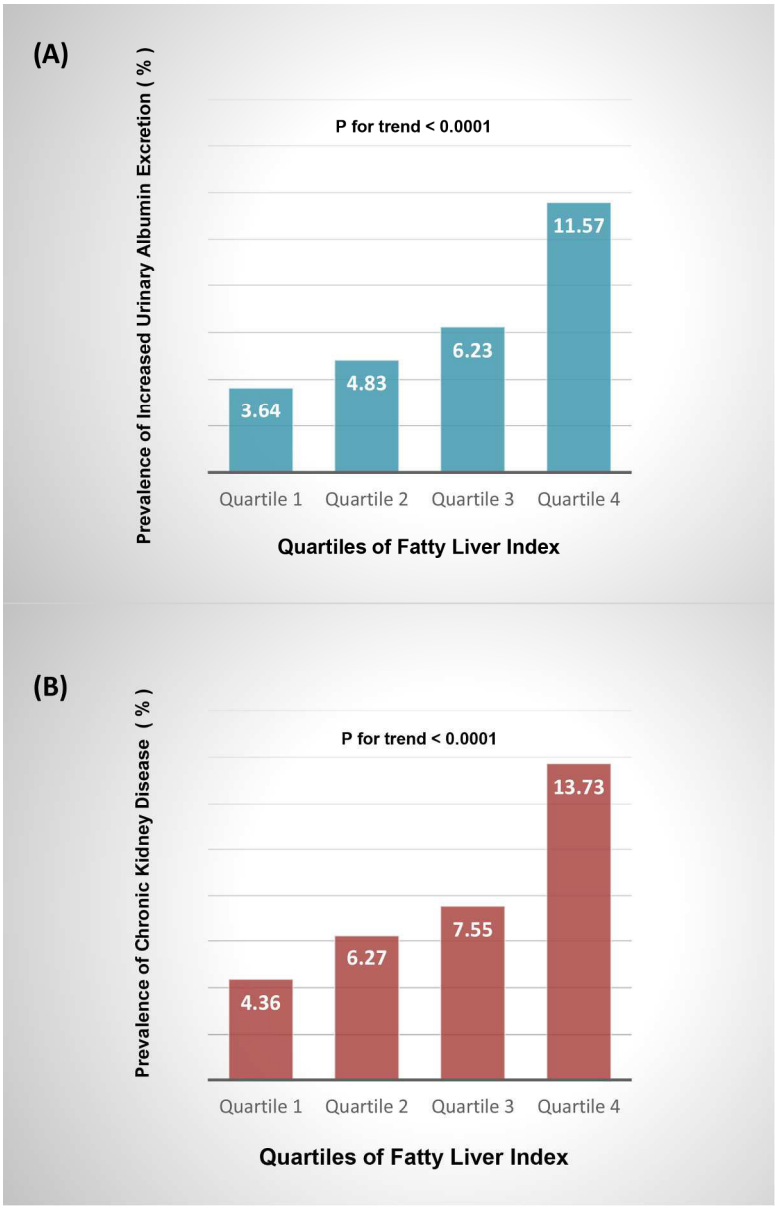

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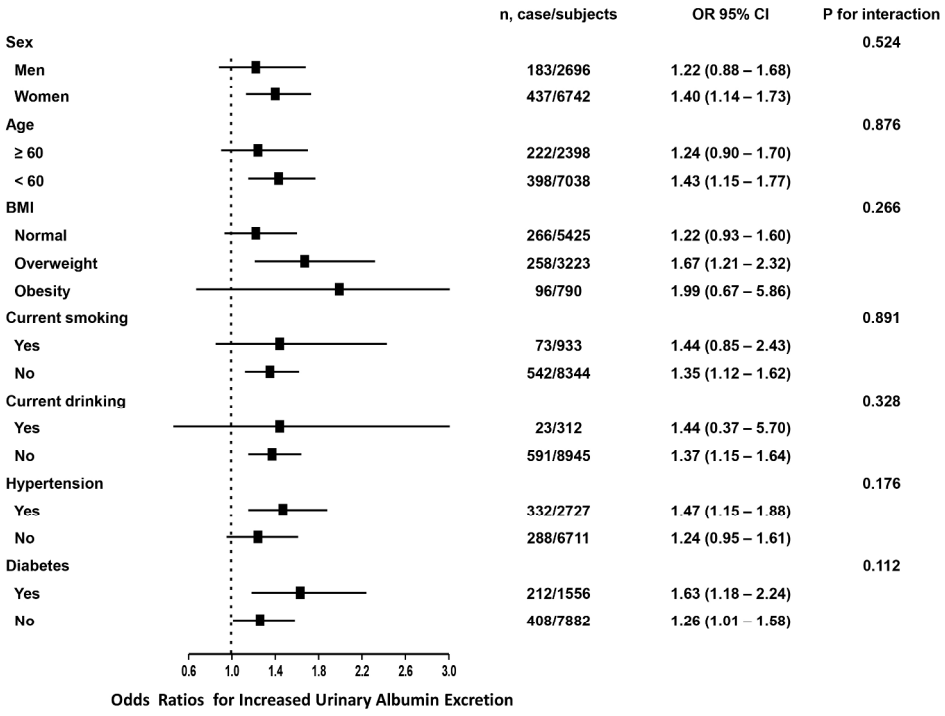

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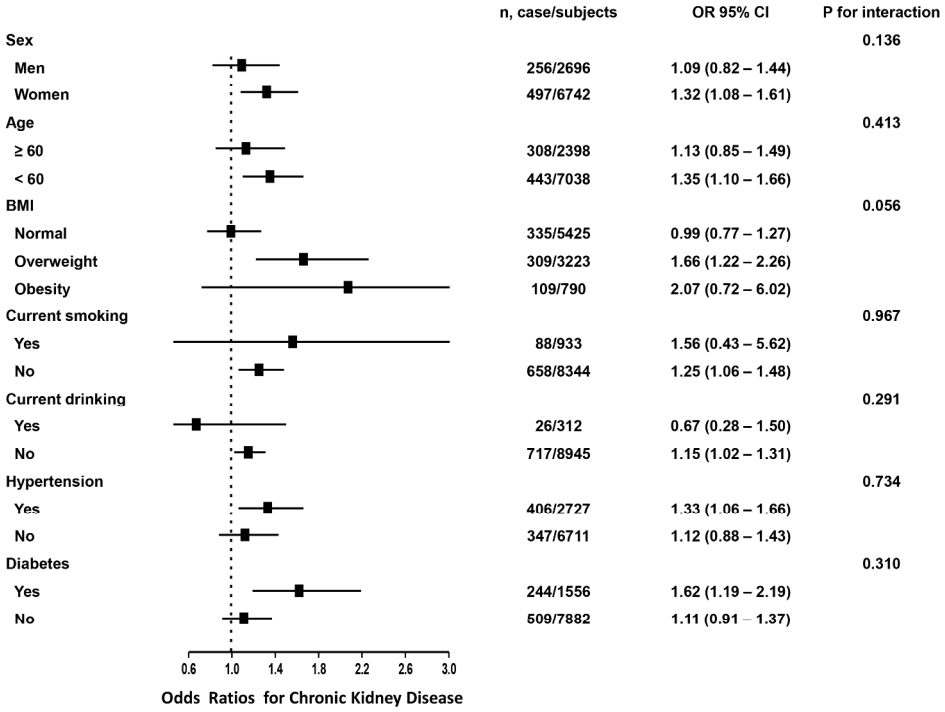

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	**L 1,2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	**L 67-91**
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	**L 105-137**
Objectives	3	State specific objectives, including any prespecified hypotheses	**L 130-137**
Methods			
Study design	4	Present key elements of study design early in the paper	**L 141-146**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	**L 141-179**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	**L 141-153**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	**L 155-207**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	**L 193-207**
Bias	9	Describe any efforts to address potential sources of bias	**L 227-242**
Study size	10	Explain how the study size was arrived at	**L 143-146**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	**L 210-219**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	**L 210-242**
		(*b*) Describe any methods used to examine subgroups and interactions	**L 236-239**
		(*c*) Explain how missing data were addressed	**N.A.**
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	**N.A.**
		(*e*) Describe any sensitivity analyses	**N.A.**
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	**L 248-256**
		(b) Give reasons for non-participation at each stage	**N.A.**
		(c) Consider use of a flow diagram	**N.A.**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	**L 248-262**
		(b) Indicate number of participants with missing data for each variable of	**L 248-**

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		interest	**292**
Outcome data	15*	Report numbers of outcome events or summary measures	**L 264-283**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	**L 264-283**
		(*b*) Report category boundaries when continuous variables were categorized	**L 210-219**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	**N.A.**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**L 285-292**
Discussion			
Key results	18	Summarise key results with reference to study objectives	**L 295-303**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**L 374-403**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**L 329-373**
Generalisability	21	Discuss the generalisability (external validity) of the study results	**L 377-381**
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	**L 47-59**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Fatty liver index, albuminuria and the association with chronic kidney disease: a population-based study in China

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Fatty liver index, albuminuria and the association with chronic kidney disease: a population-based study in China

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All authors believe that the manuscript represents valid work and have reviewed and approved the final version. The work has not been published previously, and not under consideration for publication elsewhere, in part or in whole.

The author contribution lists

Conceived and designed the experiments: Y. L. and K. S.

Performed the experiments: F. L., Y. Q., W. F., C. C., K. S. and D. L.

Analyzed the data: K. S. and M. R.

Wrote the manuscript: K.S. and D. L.

Data Sharing Statement

The work described was original research that has not been published previously, and not under consideration for publication elsewhere, in part or in whole. All authors believe that the manuscript represents valid work and have reviewed and approved the final version. Main document data and additional unpublished data from the study are available by sending Email to lizyhenu@163.com with proper purposes.

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Conflict of interests

The authors have declared that no competing interests exist.

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ABSTRACT

Objectives: The effects of lipid metabolism disorder on the renal damage have drawn much attention. By using the fatty liver index (FLI) as a validated indicator of hepatic steatosis, this study aims at provide insight about the possible links between fatty liver and development of chronic kidney disease (CKD).

Setting: hospital.

Participants: We performed a population-based study in 9,436 subjects aged 40 years or older.

Primary and secondary outcome measures: FLI is calculated by using an algorithm based on body mass index (BMI), waist circumference (WC), triglycerides (TG) and γ-glutamyltransferase (γ-GGT). Increased urinary albumin excretion was defined according to the urinary albumin-to-creatinine ratio ranges greater or equal than 30 mg/g. CKD was defined as estimated glomerular filtration rate (eGFR) less than 60 mL/min per 1.73 m² or presence of albuminuria.

Results: There were 620 (6.6%) subjects categorized as increased urinary albumin excretion and 753 (8.0%) subjects categorized as CKD. Participants with higher FLI had increased age, blood pressure, low-density lipoprotein cholesterol, fasting plasma glucose, fasting insulin and decreased eGFR level. Prevalence of increased urinary albumin excretion and CKD tended to increase with the elevated FLI quartiles. In logistic regression analysis, compared with subjects in the lowest quartile of FLI, the adjusted odds ratios (ORs) in the highest quartile was 2.30 [95% confidence interval

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(CI), 1.36 - 3.90] for increased urinary albumin excretion and 1.93 (95% CI, 1.18 - 3.15) for CKD.

Conclusion: Hepatic steatosis evaluating by FLI is independently associated with increased urinary albumin excretion and prevalence of CKD in middle-aged and elderly Chinese.

Keywords: Fatty liver index; Hepatic steatosis; Increased urinary albumin excretion; Chronic kidney disease

Strengths and Limitations

1. The study was performed in a large population-based cohort in 9,436 Chinese subjects.
2. Findings of the study may be applied to the majority of patients in general practice with suspected hepatic steatosis.
3. Results should be interpreted cautiously due to the observational design of the current study.

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Introduction

Chronic kidney disease (CKD) has become one of the leading public health problem world-wide [1]. Recent national survey conducted between 2007 and 2010 reports that the prevalence of CKD was 10.8%, representing an estimated 119.5 million patients in China are with chronic kidney damage [2]. In addition to CKD, an increasing number of studies have provided substantial evidence of albuminuria as a risk factor for future cardiovascular events [3]. Both renal and cardiovascular diseases sharing similar traditional risk factors, such as lipid metabolism disorder, could have particularly broad implications for the outcome of cardiovascular morbidity and mortality.

Association of hepatic steatosis with CKD development and its impact on the reduction of the estimated glomerular filtration rate (eGFR) have been extensively investigated over the past decade [4]. The substantial evidence linked hepatic steatosis to the increased risk and severity of CKD, which may be a target for the prevention and treatment of the disease [5]. As a convenient scoring system for the presence of hepatic lipid deposits, the fatty liver index (FLI) is a surrogate steatosis biomarker developed in a cohort of patients from the general population [6]. Compared with other techniques for evaluating hepatic steatosis, FLI is simple to obtain as body mass index (BMI), waist circumference (WC), triglycerides (TG) and γ-glutamyltransferase (γ-GGT) are routine measurements in clinical practice. Previous studies have demonstrated that FLI could determine fatty liver disease, incident type 2 diabetes and incident hypertension with considerable accuracy [6-8]. Moreover, FLI is associated with

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insulin resistance early atherosclerosis and risk of coronary heart disease, which could help physicians early detect subjects of greater cardiovascular risk and select patients for intensified lifestyle counseling [9 10].

Clarifying the association of FLI with albuminuria and prevalent CKD would probably shed light on the prevention and preemptive treatment of related diseases. Recently, a cross-sectional study was conducted to investigate the association between FLI and CKD by recruiting adults undergoing a health check-up [11]. However, by including only 731 subjects, the study did not evaluate the association between FLI and albuminuria, either. Therefore, we analyzed data from a community-based Chinese population to comprehensively look into the relationship of FLI with both increased urinary albumin excretion and CKD.

Subjects and methods

Study population and design

We performed a cross-sectional study in a community in Guangzhou, China from June to November, 2011. The study population was from the REACTION study and details of this study have been published previously [12-14]. During the recruiting phase, a total of 10,104 residents aged 40 years or older were invited to participate by examination notices or home visits. In total, 9,916 subjects signed the consent form and agreed to participate in the survey. The participation rate was 98.1%. The subjects who failed to provide information (BMI: n=206; WC: n=62; TG: n=23; γ-GGT: n=38; or urinary albumin-to-creatinine ratio [ACR]: n=149) were excluded from the

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analyses. Accordingly, a total of 9,438 eligible individuals were included in the final data analyses. The study protocol was approved by the Institutional Review Board of the Sun Yat-sen Memorial Hospital affiliated to Sun Yat-sen University and was in accordance with the principle of the Helsinki Declaration II. Written informed consent was obtained from each participant before data collection.

Clinical and biochemical measurements

We collected information on lifestyle factors, sociodemographic characteristics and family history by using a standard questionnaire. Smoking or drinking habit was classified as 'never', 'current' (smoking or drinking regularly in the past 6 months) or 'ever' (cessation of smoking or drinking more than 6 months) [15]. A short form of the International Physical Activity Questionnaire (IPAQ) was used to estimate physical activity at leisure time by adding questions on frequency and duration of moderate or vigorous activities and walking [16]. Separate metabolic equivalent hours per week (MET-h/week) were calculated for evaluation of total physical activity.

All participants completed anthropometrical measurements are with the assistance of trained staff by using standard protocols. Three times consecutively blood pressure measurements by the same observer in a 5-minute interval were obtained by an automated electronic device (OMRON, Omron Company, China). The average of three measurements of blood pressure was used for analysis. Body height and body weight were recorded to the nearest 0.1 cm and 0.1 kg while participants were wearing light indoor clothing without shoes. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Obesity was defined as BMI

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equal or greater than 28 and overweight was defined as BMI equal or greater than 24 and less than 28 [17]. WC was measured at the umbilical level with participant in standing position, at the end of gentle expiration.

Venous blood samples were collected for laboratory tests after an overnight fasting of at least 10 hours. Measurement of fasting plasma glucose (FPG), fasting serum insulin, TG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine, γ-GGT, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was done using an autoanalyser (Beckman CX-7 Biochemical Autoanalyser, Brea, CA, USA).

As surrogate marker of hepatic steatosis, FLI was analyzed based on BMI, WC, TG, and γ-GGT, which has been validated against liver ultrasound in the general population and has been proven accurate in detecting fatty liver [6 10]. FLI is calculated as: $FLI = (e^{0.953 * \log e(TG) + 0.139 * BMI + 0.718 * \log e(GGT) + 0.053 * WC - 15.745}) / (1 + e^{0.953 * \log e (TG) +0.139 * BMI + 0.718 * \log e (GGT) + 0.053 * WC - 15.745}) * 100$. The abbreviated Modification of Diet in Renal Disease (MDRD) formula recalibrated for Chinese population was used to calculate estimated glomerular filtration rate (GFR) expressed in mL/min per 1.73 m^2 using a formula of $eGFR = 175 \times [\text{serum creatinine} \times 0.011]^{-1.234} \times [\text{age}]^{-0.179} \times [0.79 \text{ if female}]$, where serum creatinine was expressed as μmol/L [18]. Diabetes was diagnosed according to the 1999 World Health Organization diagnostic criteria [19].

Definition of increased urinary albumin excretion, chronic kidney disease and non-alcoholic fatty liver disease (NAFLD)

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Definitions of abnormalities in albumin excretion were according to the latest guidelines of American Diabetes Association's Standards of Medical Care [20]. The first morning spot urine samples were collected for assessing the ACR. Urine albumin and creatinine were measured by chemiluminescence immunoassay (Siemens Immulite 2000, United States) and the Jaffe' s kinetic method (Biobase-Crystal, Jinan, China) on the automatic analyzer, respectively. ACR was calculated by dividing the urinary albumin concentrations by the urinary creatinine concentrations and expressed in mg/g. The primary and secondary outcome measures were increased urinary albumin excretion and chronic kidney disease (CKD), respectively. Increased urinary albumin excretion was defined according to the ACR ranges greater or equal than 30 mg/g. Chronic kidney disease (CKD) was defined as eGFR less than 60 mL/min per 1.73 m^2 or presence of albuminuria (ACR greater or equal than 30 mg/g). The optimal cutoff value of FLI for predicting NAFLD was 30 in Asian populations [21]. Therefore, we classified the study population in non-current drinking group into NAFLD group (FLI $\geq$ 30) and non-NAFLD group (FLI < 30).

Statistical analysis

Statistical analysis was performed using SAS version 9.2 (SAS Institute Inc, Cary, NC, USA). Continuous variables were presented as means ± standard deviation (SD) except for skewed variables, which were presented as medians (interquartile ranges). Categorical variables were expressed as numbers (proportions). FLI, FPG, TG, ALT, AST, γ-GGT and MET-h/week were logarithmically transformed before analysis due

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to a non-normal distribution. FLI was presented as quartiles and linear regression analysis was used to test for trend across groups. Differences among groups were tested by one-way ANOVA and *post hoc* comparisons were performed by using Bonferroni correction. Comparisons between categorical variables were performed with the χ^2 test.

Pearson's correlations were performed to test the correlations between FLI and the risk factors for kidney disease. Variables significant at $P < 0.20$ in Pearson's correlations were put into the multivariate stepwise linear regression models to identify factors that independently associated with FLI. We analyzed the impact of FLI on the prevalence of increased urinary albumin excretion and CKD. The unadjusted and multivariate-adjusted logistic regression analysis was used to assess the risk of prevalent increased urinary albumin excretion and CKD in relation to each quartile increase in FLI level. Variables considered as potential covariates and significant in the stepwise linear regression were put into multivariate-adjusted logistic regression analysis. Model 1 is unadjusted. Model 2 is adjusted for age. Model 3 is adjusted for age, sex, current smoking status, current drinking status, physical activity, systolic blood pressure (SBP), diastolic blood pressure (DBP), LDL-C, fasting insulin, ALT and AST. Model 4 is adjusted for age, sex, BMI, WC, current smoking status, current drinking status, physical activity, systolic blood pressure (SBP), diastolic blood pressure (DBP), TG, LDL-C, fasting insulin, ALT, AST and γ-GGT. Odds ratios (OR) and the corresponding 95% confidence intervals (95% CI) were calculated. Relationship of FLI level with albuminuria and CKD were also

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explored in subgroups stratified by gender (men/women), age (≥ 60/< 60 years), degree of obesity (normal/overweight/obesity), current smoking (yes/no), current drinking (yes/no), hypertension (yes/no) and diabetes (yes/no). Tests for interaction were performed with including simultaneously each strata factor, the quartiles of FLI level and the respective interaction terms (strata factor multiplied by quartiles of FLI level) in the models.

All statistical tests were two-sided, and a P value < 0.05 was considered statistically significant.

Results

Clinical characteristics of the study population

Among the 9,436 enrolled individuals, the mean age was 55.9 ± 8.0 years. The median FLI was 19.1 with interquartile range 8.6 to 37.4. There were 620 (6.6%) subjects categorized as increased urinary albumin excretion and 753 (8.0%) subjects categorized as CKD, respectively. Table 1 shows the clinical and biochemical characteristics of the participants according to FLI quartiles. Participants with higher FLI level had elevated age, BMI, WC, SBP, DBP, TG, TC, LDL-C, FPG, fasting insulin, ALT, AST, γ - GGT and higher proportions of current smokers and current drinkers (all P for trend < 0.0001). Those with higher FLI level also associated with decreased HDL-C and eGFR (all P for trend < 0.0001).

Associations between FLI and metabolic risk factors

Analysis of Pearson's correlation showed that age, sex, BMI, WC, SBP, DBP, TG,

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TC, HDL-C, LDL-C, FPG, fasting insulin, ALT, AST, γ-GGT and eGFR were significantly correlated with FLI level. Further multivariate stepwise linear regression showed that age, sex, BMI, WC, SBP, DBP, TG, LDL-C, fasting insulin, ALT, AST and γ-GGT were independent determinants for FLI level (Table 2).

Associations of FLI with increased urinary albumin excretion and CKD

As shown in Figure.1A, from the lowest quartile to the highest quartile of FLI level, the prevalence of increased urinary albumin excretion was 3.64%, 4.83%, 6.23% and 11.57%, respectively (P for trend < 0.0001). Strikingly, the prevalence of CKD also tended to increase with the elevated FLI quartile (Figure.1B, P for trend < 0.0001). As shown in Table 3, compared with participants in quartile 1 of FLI, univariate logistic regression analysis showed that participants in quartile 2, quartile 3 and quartile 4, respectively, have a significant correlation with increased odds of increased urinary albumin excretion and CKD (all P for trend < 0.0001). In multivariate logistic regression analyses (Model 3), the ORs of increased urinary albumin excretion for increasing FLI quartiles were 1.00 (reference), 0.96 (95% CI 0.66 - 1.39), 1.17 (95% CI 0.77 - 1.77) and 2.30 (95% CI 1.36 - 3.90). Similarly, the ORs of CKD for increasing FLI quartiles in Model 3 were 1.00 (reference), 1.00 (95% CI 0.71 - 1.40), 1.03 (95% CI 0.70 - 1.51) and 1.93 (95% CI 1.18 - 3.15), respectively (Table 3). The prevalence of increased urinary albumin excretion was 51.6% and 29.6% in FLI established NAFLD and non-NAFLD group (P < 0.0001). Similar trends were detected in the prevalence of CKD (NAFLD group: 49.9%; non-NAFLD group: 31.5%, P < 0.0001). Compared with participants in the non-NAFLD group,

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those in NAFLD group had higher prevalence of increased urinary albumin excretion (OR 1.58, 95 % CI 1.18 - 2.13) and CKD (OR 1.39, 95 % CI 1.05 - 1.82) in multivariate logistic regression analyses.

Subgroups analysis of FLI with increased urinary albumin excretion and CKD

As shown in Figure. 2 & 3, the associations of FLI level with increased urinary albumin excretion and CKD were not consistently the same in subgroups analyses. Significant relationship of FLI level with both increased urinary albumin excretion and CKD were detected in women, younger subjects (age less than 60 years), overweight subjects, non-current smokers, non-current drinkers and in those with hypertension or with diabetes (all $P < 0.05$). In the subgroups analysis, no statistically significance of interaction term between quartiles of FLI and each strata factor was detected.

Discussion

We evaluated the association between hepatic steatosis and kidney disease in a large population of middle-age Chinese subjects from the REACTION study. Presence of fatty liver assessed by FLI was associated with increased urinary albumin excretion and reduction of the eGFR in the present study. The association was independent of potential confounding risk factors. To our current knowledge, this is the largest population-based study to explore the association of FLI with both albuminuria and CKD in Asian population. Early intervention is of great importance for albuminuria and CKD, the present findings may just give insights into lipid

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metabolism for prevention and early detection of the diseases.

The problem of obesity and NAFLD are now increasingly recognized in the Asian population. Prevalence of obesity was 7.9% (8.4% in males and 7.6% in females) in southern China, which has increased dramatically over the past several decades [22]. There is a strong correlation between established obesity and incidence of NAFLD. Pooled prevalence of NAFLD diagnosed by ultrasound, computed tomography scan and magnetic resonance was estimated to be 27.4% in subjects aged over 30 years from Asian countries [23]. Even among the non-obese Chinese, 8.9% developed NAFLD in five years from 2006 to 2011 [24]. Therefore, early and accurate diagnosis of NAFLD is of great importance. The best method for an accurate assessment and diagnosis of hepatic steatosis is histologic analysis of biopsies [25]. However, it is uneconomical to conduct liver biopsies especially by the fact of our large sample population. Hepatic ultrasonic examination is widely used in clinical practice and epidemiological studies in detecting fatty infiltration of the liver [26 27]. However, the noninvasive technique is not sensitive enough to detect mild steatosis and does not allow precise quantification of severity of steatosis in hepatic tissue [28].

As another surrogate marker of histological fatty liver, FLI is defined as the accumulation of excessive liver fat [29]. Based on the former researches, FLI has been proven accurate in detecting fatty liver against liver ultrasound and demonstrating the presence of hepatic fat against magnetic resonance spectroscopy [6 9 10 21]. The superiority of this non-invasive assessment techniques is that a higher score will indicate a higher degree of steatosis in hepatic tissue. However, optimal cut-off point

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of the FLI for evaluating liver fatty infiltration should be considered as it varied according to the study population [21 30]. Originally, FLI＞60 was suggested to rule in NAFLD in Caucasian subjects. However, the optimal cut-off value of FLI for predicting NAFLD was different in Asian populations. In one recent study, Huang et al. [21] found that FLI could accurately identify NAFLD and the optimal cut-off point was 30 in middle-aged and elderly Chinese. FLI could also accurately identify ultrasonography fatty liver in a large scale population in Taiwan but with different optimal cut-off values, while an FLI＞35 for males and＞20 for females rule in NAFLD in their study [30]. Through the results of our research in Chinese subjects, further studies are therefore needed to externally discuss the optimal cut-off point of the FLI for predicting hepatic steatosis.

Detection and prevention of kidney disease progression and urinary albumin excretion is difficult to process in the early stage. Dyslipidemia is increasingly recognized as important pathogenic mechanism in deterioration of renal function. Recently, we conducted a clinical investigation to assess the associations of routine lipid measures with kidney disease in the same cohort. In the study, discordant associations of lipid parameters with renal insufficiency was detected while TG to HDL-C ratio is a better marker for evaluating increased urinary albumin excretion and CKD [31]. As one of the phenotype of dyslipidemia, the pathogeneses of hepatic steatosis is closely related to kidney disease with regard to insulin resistance and chronic inflammation [32]. Hepatokines, which are proteins secreted by hepatocytes, have been found to link to the induction of metabolic phenotypes through inter-organ

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communication based on recent studies [33]. Because of the high prevalence and burden of the fatty liver disease, it is important to identify which patients are most likely to be exposed to early stage renal injury [23]. Consequently, we closely monitor the association of the hepatic steatosis predict by FLI with prevalent increased urinary albumin excretion and CKD.

Consistent with our findings, a previous study reported that hepatic steatosis evaluated by FLI might contribute to CKD development [11]. Elevated albuminuria is well known to be associated with increased risk for early diabetes renal damage, however, the identification and classification of kidney disease was assessed only by eGFR in that study. Moreover, 731 adults that underwent routine health evaluations were included in that study and the small sample size cannot better represent the whole population. By totally including 9,438 subjects and adopting both albuminuria and eGFR for renal damage assessment, data in our study demonstrated that the FLI is associated with kidney disease, which might be an efficient screening indicator for the early prevention of related diseases in Chinese subjects. Recently, an interesting study by Giorda C et al. [34] reported that NAFLD is a dynamic condition in type 2 diabetes subjects and about 5% Italian diabetic patients entering or leaving FLI assessed NAFLD status every year. They found that male sex and established organ damage, especially kidney function, were independent risk predictors for the dynamic NAFLD condition in a longitudinal 3-year analysis. As the similarity in traditional risk factors for both NAFLD and CKD, relationship between the prevalence of earlier stages of kidney damage and the incidence of NAFLD is complex. Longitudinal observation of

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our cohort are needed to be carried out to determine whether such dynamic condition existed in the Chinese, especially in those with type 2 diabetes.

Alcohol consumption can profoundly disturb the lipid metabolism which have prominent effects on the hepatic tissue steatosis and insulin sensitivity [35]. However, potential health effects regarding alcohol consumption in this field is also worth attaching attention. A meta-analysis of intervention studies by Schrieks et al [36]. showed that moderate alcohol intake could improve insulin sensitivity by decreasing fasting insulin level in women. Recently, a prospective cohort study found that alcohol consumption was consistently inversely associated with urinary albumin excretion and the risk of developing CKD [37]. Therefore, advice concerning alcohol consumption to subjects with low-grade hepatic tissue steatosis should consider the full range of benefits and risks, especially among those who drink moderately.

Some limitations of the study must be noted. Firstly, owing to the observational design of the current study, we should cautiously interpret the present findings as no causal inference can be drawn. Further prospective studies are therefore needed to determine the precise relationship between FLI and risk of renal diseases. Secondly, by including only Chinese subjects, the results of the present study might not be representative of other ethnic groups, especially for those in the developed or undeveloped countries. To some extent, however, the present study of Chinese population was still a convenience sample and selection bias is inevitable. Thirdly, when evaluating the findings of the present study, the results should be interpreted cautiously due to possible bias from using the indirect indicator FLI to assess fatty

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liver disease. The calculated FLI may relate to various liver diseases with associated steatosis and not only NAFLD, despite the fact that metabolic disturbances make obesity related steatosis likely. The internal accuracy of FLI for evaluation hepatic steatosis should also be validated by using other techniques, before it can be employed for these purposes. Fourthly, we observed that FLI seem to play a different efficiency for kidney disease assessment in different stratifications. A significant association of FLI with increased urinary albumin excretion and CKD only detected in subjects without current alcohol consumption. Average daily alcohol intake influences the FLI and missing such data in the present study doesn't permit comparisons between and within alcoholic and nonalcoholic fatty liver disease groups. To better discriminate alcoholic fatty liver disease and non-alcoholic fatty liver disease, further studies need to clearly described the precise exposure of alcohol use by collecting histories of alcohol intake in a quantitative manner. Fifthly, viral hepatitis infection is one of the most serious infectious diseases worldwide, which can be associated with both liver and kidney disease. Recent survey data showed that the hepatitis B surface antigen and anti-hepatitis C virus–positive rates were already 6.1% and 3.0% in China. Epidemiology of viral hepatitis infection by hepatitis B virus (HBV) and hepatitis C virus (HCV) serological testing, therefore, should be also be evaluate to strength the findings of the present study [38]. Sixthly, although a spectrum of covariates was included in the adjustment, other potential mediators such as daily energy and protein intake and medicine that influence the renin-angiotensin-system of the subjects, should also be considered in the present

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study.

In conclusion, by including a large population based cohort, the present study provides evidence that increased FLI is independently associated with prevalence of albuminuria and CKD. Findings of the present study suggested us should pay more attention to albuminuria and eGFR variation in patients with dyslipidemia and fatty liver disease. Further prospective studies are necessary to verify our findings in external populations.

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Figure legends

Figure. 1 Prevalence of increased urinary albumin excretion and CKD in different quartiles of FLI levels. (A) Increased urinary albumin excretion. (B) CKD.

Figure. 2 Risk of prevalent increased urinary albumin excretion with each quartile increase of FLI levels in different subgroups.

Figure. 3 Risk of prevalent CKD with each quartile increase of FLI levels in different subgroups.

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434

435

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Table 1. Characteristics of study population by FLI quartiles

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
n (%)	2360 (25.01)	2359 (24.99)	2359 (24.99)	2360 (25.01)	
Fatty liver index	5.00 (3.27 – 6.74)	13.23 (10.76 – 15.99)	26.96 (22.74 – 31.75)	54.71 (45.40 – 68.10)	
Urinary albumin to creatinine ratio (mg/g)	7.65 (5.59 – 11.12)	8.01 (5.64 – 11.71)	8.06 (5.73 – 11.83)*	8.93 (5.96 – 15.01)*#&	< 0.0001
Age (years)	54.3 ± 7.8	55.8 ± 7.9*	56.5 ± 7.9*#	56.9 ± 8.3*#	< 0.0001
Male [n (%)]	427 (18.09)	593 (25.17)	701 (29.72)	975 (41.31)	< 0.0001
BMI (kg/m^2)	20.6 ± 2.0	22.9 ± 2.0*&	24.4 ± 2.1*#	26.8 ± 3.5*#&	< 0.0001
WC (cm)	72.0 ± 5.8	79.3 ± 5.4*&	84.1 ± 5.5*#	91.3 ± 8.5*#&	< 0.0001
SBP (mmHg)	118.6 ± 14.7	124.5 ± 15.9*&	128.4 ± 15.8*#	132.5 ± 16.1*#&	< 0.0001
DBP (mmHg)	71.2 ± 9.1	74.2 ± 9.3*&	76.5 ± 9.4*#	79.3 ± 9.8*#&	< 0.0001
Current smoking [n (%)]	169 (7.3)	202 (8.7)	227 (9.8)	335 (14.4)	< 0.0001
Current drinking [n (%)]	57 (2.5)	70 (3.0)	68 (2.9)	117 (5.1)	< 0.0001
TG (mmol/L)	0.85 (0.69 – 1.07)	1.12 (0.90 – 1.43) *&	1.49 (1.13 – 1.94) *#	2.10 (1.56 – 3.01) *#&	< 0.0001
TC (mmol/L)	4.79 ± 1.24	5.16 ± 1.22*&	5.35 ± 1.13 *#	5.54 ± 1.17*#&	< 0.0001
HDL-C (mmol/L)	1.45 ± 0.41	1.37 ± 0.35*&	1.29 ± 0.31*#	1.19 ± 0.28*#&	< 0.0001

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LDL-C (mmol/L)	2.82 ± 0.90	3.19 ± 0.94$^{*\&}$	3.31 ± 0.91$^{*\#}$	3.28 ± 0.95$^{*\#}$	< 0.0001
FPG (mmol/L)	5.23 (4.89 – 5.61)	5.33 (4.95 – 5.80) $^{*\&}$	5.47 (5.05 – 5.96) $^{*\#}$	5.73 (5.23 – 6.42) $^{*\#\&}$	< 0.0001
Fasting insulin (μIU/ml)	5.10 (3.90 – 6.50)	6.50 (5.00 – 8.40) $^{*\&}$	7.90 (6.10 – 10.30) $^{*\#}$	10.50 (7.80 – 13.70) $^{*\#\&}$	< 0.0001
ALT (U/L)	10.0 (8.0 – 14.0)	12.0 (9.0 – 16.0) $^{*\&}$	13.0 (10.0 – 17.0) $^{*\#}$	17.0 (12.0 – 24.0) $^{*\#\&}$	< 0.0001
AST (U/L)	17.0 (14.0 – 20.0)	18.0 (15.0 – 21.0) $^{*\&}$	18.0 (15.0 – 22.0) $^{*\#}$	20.0 (17.0 – 25.0) $^{*\#\&}$	< 0.0001
γ-GGT (U/L)	14.0 (11.0 – 17.0)	18.0 (14.0 – 23.0) $^{*\&}$	22.0 (17.0 – 29.0) $^{*\#}$	31.0 (23.0 – 47.0) $^{*\#\&}$	< 0.0001
Serum creatinine (μmol/L)	65.3 ± 15.5	68.8 ± 16.0$^{*\&}$	70.5 ± 16.0$^{*\#}$	74.9 ± 17.2 $^{*\#\&}$	< 0.0001
eGFR (ml/min per 1.73 m^2)	108.0 ± 25.4	102.5 ± 23.7$^{*\&}$	99.9 ± 19.6 $^{*\#}$	95.5 ± 19.5$^{**\#\&}$	< 0.0001
Physical activity (MET-h/week)	24.0 (10.5 – 49.0)	24.0 (10.5 – 45.0)	23.0 (10.5 – 42.0)	21.0 (10.5 – 42.0)*	0.006

1. Data were means ± SD or medians (interquartile ranges) for skewed variables or numbers (proportions) for categorical variables.
2. P for trend was calculated for the linear regression analysis tests across the groups. P values were for the ANOVA or χ^2 analyses across the groups.
3. *P < 0.05 compared with Quartile 1 of fatty liver index; $^{\#}$P < 0.05 compared with Quartile 2 of fatty liver index; $^{\&}$P < 0.05 compared with Quartile 3 of fatty liver index.
4. BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GGT, γ-glutamyltransferase; eGFR, estimated glomerular filtration rate.

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Table 2. Pearson's correlation and stepwise regression analysis of determinants of FLI

	r	P value	Standardized β	P value
Age (years)	0.12	< 0.0001	0.01	0.010
Sex (men=1, women=2)	-0.19	< 0.0001	-0.04	< 0.0001
BMI (kg/m^2)	0.71	< 0.0001	0.30	< 0.0001
WC (cm)	0.78	< 0.0001	0.42	< 0.0001
Physical activity (MET-h/week)	-0.02	0.060	-	-
SBP (mmHg)	0.32	< 0.0001	0.01	0.006
DBP (mmHg)	0.32	< 0.0001	0.01	0.047
TG (mmol/L)	0.68	< 0.0001	0.42	< 0.0001
HDL-C (mmol/L)	-0.26	< 0.0001	-	-
LDL-C (mmol/L)	0.21	< 0.0001	0.06	< 0.0001
FPG (mmol/L)	0.22	< 0.0001	-	-
Fasting insulin (μIU/ml)	0.40	< 0.0001	0.01	0.0002
ALT (U/L)	0.20	< 0.0001	0.05	< 0.0001
AST (U/L)	0.15	< 0.0001	-0.03	< 0.0001
γ-GGT (U/L)	0.35	< 0.0001	0.16	< 0.0001
eGFR (ml/min per 1.73 m^2)	-0.19	< 0.0001	-	-

r, correlation coefficient; β, regression coefficient.

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556

Table 3. The risk of prevalent albuminuria and CKD according to quartiles of FLI

		Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Increased urinary albumin excretion	Model 1	1	1.34 (1.01 – 1.79)	1.76 (1.34 – 2.31)	3.46 (2.70 – 4.44)	< 0.0001
	Model 2	1	1.29 (0.97 – 1.72)	1.66 (1.27 – 2.19)	3.25 (2.53 – 4.17)	< 0.0001
	Model 3	1	0.94 (0.66 – 1.33)	1.13 (0.81 – 1.59)	2.22 (1.60 – 3.07)	< 0.0001
	Model 4	1	0.96 (0.66 – 1.39)	1.17 (0.77 – 1.77)	2.30 (1.36 – 3.90)	0.001
CKD	Model 1	1	1.47 (1.13 – 1.90)	1.79 (1.39 – 2.30)	3.49 (2.77 – 4.39)	< 0.0001
	Model 2	1	1.39 (1.07 – 1.80)	1.65 (1.28 – 2.12)	3.16 (2.51 – 3.99)	< 0.0001
	Model 3	1	0.99 (0.73 – 1.36)	1.03 (0.75 – 1.40)	1.95 (1.44 – 2.64)	< 0.0001
	Model 4	1	1.00 (0.71 – 1.40)	1.03 (0.70 – 1.51)	1.93 (1.18 – 3.15)	0.012

Data are odds ratios (95% confidence interval). Participants without increased urinary albumin excretion or CKD are defined as 0 and with increased urinary albumin excretion or CKD as 1.
Model 1 is unadjusted.
Model 2 is adjusted for age.
Model 3 is adjusted for age, sex, current smoking status, current drinking status, physical activity, SBP, DBP, LDL-C, fasting insulin, ALT and AST.
Model 4 is adjusted for age, sex, BMI, WC, current smoking status, current drinking status, physical activity, SBP, DBP, TG, LDL-C, fasting insulin, ALT,

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AST and γ-GGT.

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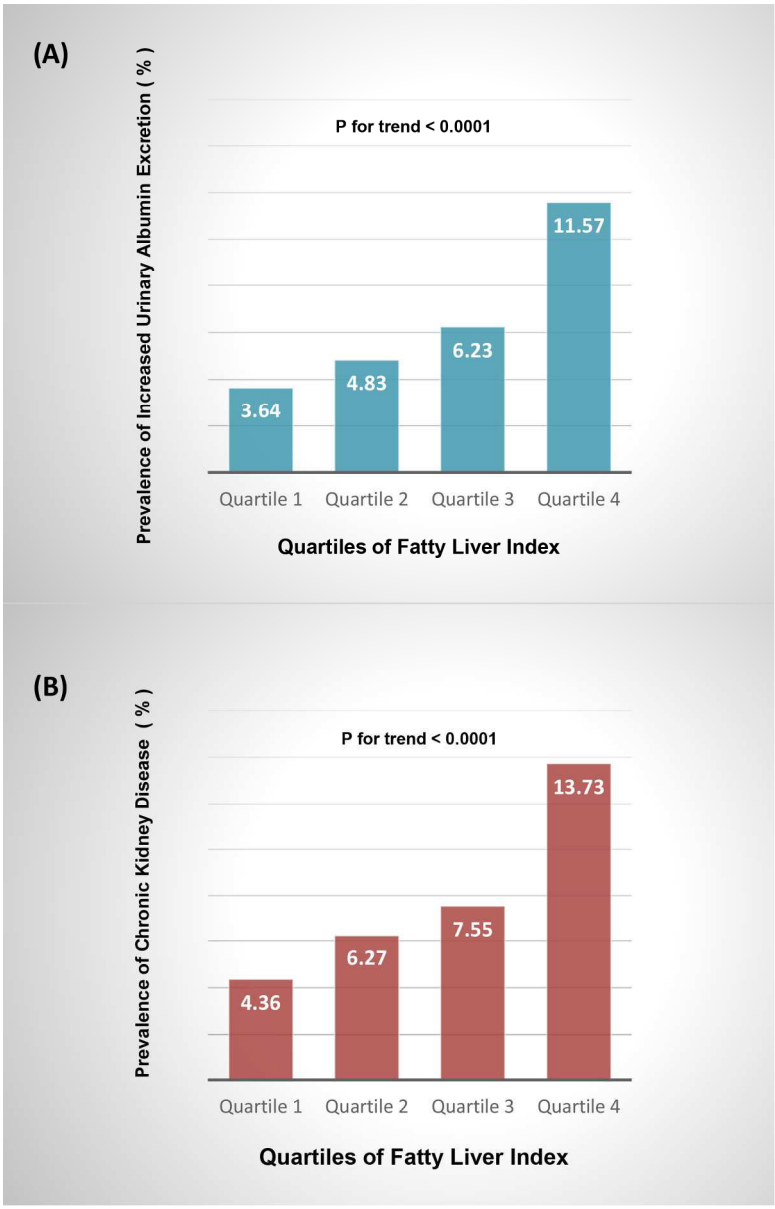

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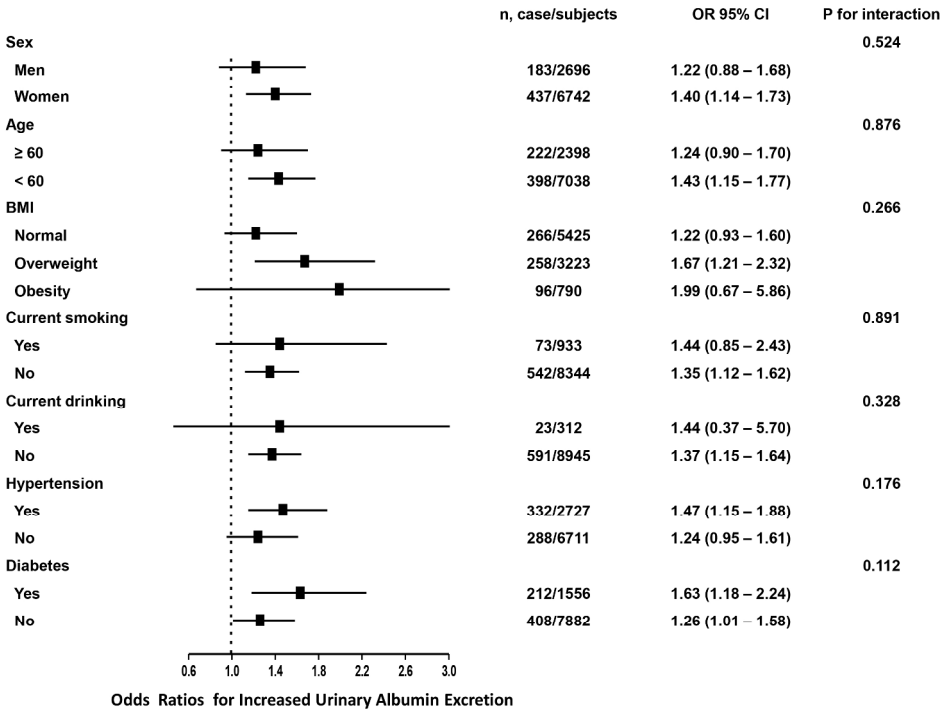

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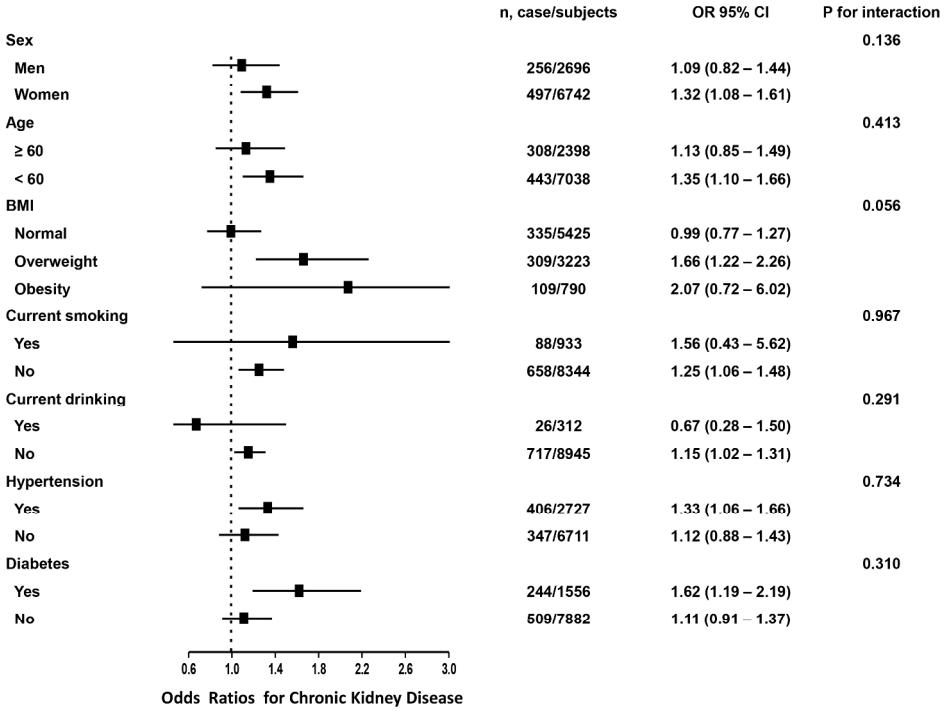

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	**L 1,2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	**L 67-91**
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	**L 105-137**
Objectives	3	State specific objectives, including any prespecified hypotheses	**L 130-137**
Methods			
Study design	4	Present key elements of study design early in the paper	**L 141-146**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	**L 141-179**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	**L 141-153**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	**L 155-207**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	**L 193-207**
Bias	9	Describe any efforts to address potential sources of bias	**L 227-242**
Study size	10	Explain how the study size was arrived at	**L 143-146**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	**L 210-219**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	**L 210-242**
		(*b*) Describe any methods used to examine subgroups and interactions	**L 236-239**
		(*c*) Explain how missing data were addressed	**N.A.**
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	**N.A.**
		(*e*) Describe any sensitivity analyses	**N.A.**
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	**L 248-256**
		(b) Give reasons for non-participation at each stage	**N.A.**
		(c) Consider use of a flow diagram	**N.A.**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	**L 248-262**
		(b) Indicate number of participants with missing data for each variable of	**L 248-**

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		interest	**292**
Outcome data	15*	Report numbers of outcome events or summary measures	**L 264-283**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	**L 264-283**
		(*b*) Report category boundaries when continuous variables were categorized	**L 210-219**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	**N.A.**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**L 285-292**
Discussion			
Key results	18	Summarise key results with reference to study objectives	**L 295-303**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**L 374-403**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**L 329-373**
Generalisability	21	Discuss the generalisability (external validity) of the study results	**L 377-381**
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	**L 47-59**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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